

Burke  
09/126816

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FILE 'CAPLUS' ENTERED AT 11:32:29 ON 16 NOV 1999  
L1 2453 S RAS(S) (P21 OR PROTO?)  
L2 1 S L1 AND (TOXIN(S) LT OR LETHAL TOXIN)  
L3 22 S RAS AND (TOXIN(S) LT OR LETHAL TOXIN)  
L4 20 S L3 AND SORDELL?  
L5 20 S L2 OR L4

- key terms

L5 ANSWER 1 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1999:583555 CAPLUS  
DOCUMENT NUMBER: 131:209119  
TITLE: Toxicologically active fragments of  
lethal toxin from Clostridium  
sordellii and their application in  
immunotoxins  
INVENTOR(S): Aktories, Klaus; Hofmann, Fred  
PATENT ASSIGNEE(S): Albert-Ludwigs-Universitaet Freiburg, Germany  
SOURCE: Ger. Offen., 14 pp.  
CODEN: GWXXBX  
DOCUMENT TYPE: Patent  
LANGUAGE: German  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

| PATENT NO.  | KIND | DATE     | APPLICATION NO.  | DATE     |
|-------------|------|----------|------------------|----------|
| DE 19802569 | A1   | 19990909 | DE 1998-19802569 | 19980123 |

AB Fragment 1-546 of *C. sordellii* lethal toxin and an immunotoxin comprising this protein fused to a cell-binding moiety, such as a tumor cell-binding antibody or antibody fragment, are disclosed. The immunotoxin may addnl. contain a translocation signal, e.g., the translocation domain of *Pseudomonas* exotoxin A or of the *Clostridium* C2 toxin. The 1-546 fragment of the *C. sordellii* lethal toxin was found to have higher glucosyltransferase activity with Ras as substrate than did the wild-type lethal toxin.

L5 ANSWER 2 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1999:486949 CAPLUS  
DOCUMENT NUMBER: 131:238263  
TITLE: Rundown of somatodendritic N-methyl-D-aspartate (NMDA) receptor channels in rat hippocampal neurones: evidence for a role of the small GTPase RhoA  
AUTHOR(S): Norenberg, Wolfgang; Hofmann, Fred; Illes, Peter; Aktories, Klaus; Meyer, Dieter K.  
CORPORATE SOURCE: Department of Pharmacology, Albert-Ludwigs-University, Freiburg, D-79104, Germany  
SOURCE: Br. J. Pharmacol. (1999), 127(5), 1060-1063  
Searcher : Shears 308-4994

09/126816

CODEN: BJPCBM; ISSN: 0007-1188

PUBLISHER: Stockton Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Actin filament (F-actin) depolymn. leads to the use-dependent rundown of N-methyl-D-aspartate (NMDA) receptor activity in rat hippocampal neurons. Depolymn. is promoted by Ca<sup>2+</sup> which enters the cells via NMDA receptor channels. The ras homolog (Rho) GTPases (RhoA, Rac1 and Cdc42) promote actin polymn. and thus control the actin cytoskeleton. We have investigated, by means of the whole-cell patch clamp technique, whether the actin fibers which interact with NMDA receptors are controlled by Rho GTPases. In the presence of intracellular ATP which attenuates rundown, the C3 toxin from Clostridium (C.) botulinum was used to inactivate RhoA. Indeed, it enhanced the use-dependent rundown of NMDA-evoked inward currents to a level similar to that obtained in the absence of ATP. Lethal toxin from Clostridium sordellii which inactivates Rac1 and Cdc42 lacked this effect. We suggest that the function of somatodendritic NMDA receptor channels in rat hippocampal neurons can be modulated by RhoA via its action on F-actin.

L5 ANSWER 3 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1999:351907 CAPLUS  
DOCUMENT NUMBER: 131:98722  
TITLE: G-protein-stimulated phospholipase D activity is inhibited by lethal toxin from Clostridium sordellii in HL-60 cells  
AUTHOR (S): El Hadj, Noomen Ben; Popoff, Michel R.; Marvaud, Jean-Christophe; Payrastre, Bernard; Boquet, Patrice; Geny, Blandine  
CORPORATE SOURCE: INSERM U332, ICGM, Paris, 75014, Fr.  
SOURCE: J. Biol. Chem. (1999), 274(20), 14021-14031  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Lethal toxin (LT) from Clostridium sordellii has been shown in HeLa cells to glucosylate and inactivate Ras and Rac and, hence, to disorganize the actin cytoskeleton. In the present work, we demonstrate that LT treatment provokes the same effects in HL-60 cells. We show that guanosine 5'-O-(3-thiotriphosphate)-stimulated phospholipase D (PLD) activity is inhibited in a time- and dose-dependent manner after an overnight treatment with LT. A similar dose response to the toxin was found when PLD activity was stimulated by phorbol 12-myristate 3-acetate via the protein kinase C pathway. The toxin effect on

Searcher : Shears 308-4994

09/126816

actin organization seemed unlikely to account directly for PLD inhibition as cytochalasin D and iota toxin from *Clostridium perfringens* E disorganize the actin cytoskeleton without modifying PLD activity. However, the enzyme inhibition and actin cytoskeleton disorganization could both be related to a major decrease obsd. in phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2). Likely in a relationship with this decrease, recombinant ADP-ribosylation factor, RhoA, Rac, and RalA were not able to reconstitute PLD activity in Lt-treated cells permeabilized and depleted of cytosol. Studies of phosphoinositide kinase activities did not allow us to attribute the decrease in PtdIns(4,5)P2 to inactivation of PtdIns4P 5-kinase. LT was also found to provoke a major inhibition in phosphatidylinositol 3-kinase that could not account for the inhibition of PLD activity because wortmannin, at doses that fully inhibit phosphatidylinositol 3-kinase, had no effect on the phospholipase activity. Among the three small G-proteins, Ras, Rac, and RalA, inactivated by LT and involved in PLD regulation, inactivation of Ral proteins appeared to be responsible for PLD inhibition as **LT toxin** (strain 9048) unable to glucosylate Ral proteins did not modify PLD activity. In HL-60 cells, LT treatment appeared also to modify cytosol components in relationship with PLD inhibition as a cytosol prep. from LT-treated cells was less efficient than one from control HL-60 cells in stimulating PLD activity. Phosphatidylinositol transfer proteins involved in the regulation of polyphosphoinositides and ADP-ribosylation factor, a major cytosolic PLD activator in HL-60 cells, were unchanged, whereas the level of cytosolic protein kinase C. $\alpha$  was decreased after LT treatment. We conclude that in HL-60 cells, **lethal toxin** from *C. sordellii*, in inactivating small G-proteins involved in PLD regulation, provokes major modifications at the membrane and the cytosol levels that participate in the inhibition of PLD activity. Although Ral appeared to play an essential role in PLD activity, we discuss the role of other small G-proteins inactivated by LT in the different modifications obsd. in HL-60 cells.

L5 ANSWER 4 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1999:350413 CAPLUS

DOCUMENT NUMBER: 131:142850

TITLE: Effects of cytotoxic necrotizing factor 1 and **lethal toxin** on actin

cytoskeleton and VE-cadherin localization in human endothelial cell monolayers

AUTHOR(S): Vouret-Craviari, Valerie; Grall, Dominique; Flatau, Gilles; Pouyssegur, Jacques; Boquet, Patrice; Van Obberghen-Schilling, Ellen

CORPORATE SOURCE: Centre de Biochimie, CNRS UMR 6543, Nice, 06108, Fr.

Searcher : Shears 308-4994

09/126816

SOURCE: Infect. Immun. (1999), 67(6), 3002-3008  
CODEN: INFIBR; ISSN: 0019-9567

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Integrity of the vascular endothelium is largely dependent on endothelial cell shape and establishment of intercellular junctions. Certain pathogenic bacterial toxins alter the cytoskeletal architecture of intoxicated cells by modulating the GTPase activity of p21 Rho family proteins. In the present study, the authors have analyzed the effect of Rho-directed toxins on the actin cytoskeleton and monolayer integrity of endothelial cells. *Escherichia coli* cytotoxic necrotizing factor 1 (CNF1) activated Rho in human umbilical vein endothelial cells (HUVEC). In confluent monolayers, CNF1 treatment induced prominent stress fiber formation without modifying peripheral localization of VE-cadherin, a specific marker of vascular endothelial cell adherens junctions. Further, Rho activation with CNF1 blocked thrombin-induced redistribution of VE-cadherin staining and gap formation in HUVEC monolayers. Inhibition of Rho by prolonged treatment of cells with C3 exoenzyme (*Clostridium botulinum*) eliminated actin stress fibers without disrupting the continuity of VE-cadherin staining, indicating that Rho-dependent stress fibers are not required for maintaining this adhesion receptor at sites of intercellular contact. **Lethal toxin** (*Clostridium sordellii*), an inhibitor of Rac as well as Ras and Rap, potently disrupted the actin microfilament system and monolayer integrity in HUVEC cultures.

L5 ANSWER 5 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1999:208526 CAPLUS

DOCUMENT NUMBER: 131:2919

TITLE: Ras family proteins: new players involved in the diplotene arrest of *Xenopus* oocytes

AUTHOR(S): Jessus, Catherine; Rime, Helene; Ozon, Rene

CORPORATE SOURCE: Laboratoire de Physiologie de la Reproduction,  
Inra/CNRS ESA 7080, Universite

PIERRE-ET-MARIE-CURIE, Paris, 75252, Fr.

SOURCE: Biol. Cell (1998), 90(8), 573-583

CODEN: BCELDF; ISSN: 0248-4900

PUBLISHER: Editions Scientifiques et Medicales Elsevier

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review, with 78 refs. Oogonia undergo numerous mitotic cell cycles before completing the last DNA replication and entering the meiotic prophase I. After chromosome pairing and chromatid exchanges between paired chromosomes, the oocyte I remains arrested at the diplotene stage of the 1st meiotic prophase. Oocyte growth then occurs independently of cell division; indeed, during this

Searcher : Shears 308-4994

09/126816

growth period, oocytes (4n DNA) are prevented from completing the meiotic divisions. How is the prophase arrest regulated. One of the players of the prophase block is the high level of intracellular cAMP, maintained by an active adenylate cyclase. By using **lethal toxin** from *Clostridium sordellii* (LT), a glucosyl-transferase that glucosylates and inactivates small G proteins of the **Ras** subfamily, we have shown that inhibition of either **Ras** or Rap or both proteins is sufficient to release the prophase block of *Xenopus* oocytes in a cAMP-dependent manner. The implications of **Ras** family proteins as new players involved in the prophase arrest of *Xenopus* oocytes will be discussed here.

L5 ANSWER 6 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1999:62268 CAPLUS

DOCUMENT NUMBER: 130:206160

TITLE: Inhibition of small G proteins by *Clostridium sordellii* lethal toxin

AUTHOR(S): activates cdc2 and MAP kinase in *Xenopus* oocytes  
Rime, Helene; Talbi, Nabila; Popoff, Michel R.;  
Suziedelis, Kestutis; Jessus, Catherine; Ozon,  
Rene

CORPORATE SOURCE: Laboratoire de Physiologie de la Reproduction,  
INRA/ESA-CNRS 7080, Universite Pierre et Marie  
Curie, Paris, 75252, Fr.

SOURCE: Dev. Biol. (1998), 204(2), 592-602  
CODEN: DEBIAO; ISSN: 0012-1606

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The **lethal toxin** (LT) from *Clostridium sordellii* is a glucosyltransferase that modifies and inhibits small G proteins of the **Ras** family, **Ras** and Rap, as well as Rac proteins. LT induces cdc2 kinase activation and germinal vesicle breakdown (GVBD) when microinjected into full-grown *Xenopus* oocytes. Toxin B from *Clostridium difficile*, that glucosylates and inactivates Rac proteins, does not induce cdc2 activation, indicating that proteins of the **Ras** family, **Ras**-and(or) Rap, neg. regulate cdc2 kinase activation in *Xenopus* oocyte. In oocyte exts., LT catalyzes the incorporation of [<sup>14</sup>C]glucose into a group of proteins of 23 kDa and into 1 protein of 27 kDa. The 23-kDa proteins are recognized by anti-Rap1 and anti-Rap2 antibodies whereas the 27-kDa protein is recognized by several anti-**Ras** antibodies and probably corresponds to K-**Ras**. Microinjection of LT into oocytes together with UDP-[<sup>14</sup>C]glucose results in a glucosylation pattern similar to the in vitro glucosylation, indicating that the 23- and 27-kDa proteins are in vivo substrates of LT. In vivo time-course anal. reveals that the 27-kDa protein glucosylation is completed within 2 h, well

Searcher : Shears 308-4994

09/126816

before cdc2 kinase activation, whereas the 23-kDa proteins are partially glucosylated at GVBD. This observation suggests that the 27-kDa **Ras** protein could be the *in vivo* target of LT allowing cdc2 kinase activation. Interestingly, inactivation of **Ras** proteins does not prevent the phosphorylation of c-Raf1 and the activation of MAP kinase that occurs normally around GVBD.

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L5 ANSWER 7 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:691068 CAPLUS

DOCUMENT NUMBER: 130:122632

TITLE: Activation of a  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  current in mouse fibroblasts by lysophosphatidic acid requires a pertussis toxin-sensitive G protein and **Ras**

AUTHOR(S): Repp, Holger; Koschinski, Andreas; Decker, Katrin; Dreyer, Florian

CORPORATE SOURCE: Rudolf-Buchheim-Institut fur Pharmakologie, Justus-Liebig-Universitat Giessen, Frankfurter Strasse 107, Giessen, D-35392, Germany

SOURCE: Naunyn-Schmiedeberg's Arch. Pharmacol. (1998), 358(5), 509-517

CODEN: NSAPCC; ISSN: 0028-1298

PUBLISHER: Springer-Verlag

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Lysophosphatidic acid (LPA) is a bioactive lipid that acts through G protein-coupled plasma membrane receptors and mediates a wide range of cellular responses. Here, we report that LPA activates a  $\text{K}^+$  current in NIH3T3 mouse fibroblasts that leads to membrane hyperpolarization. The activation occurs with an EC50 value of 1.7 nM LPA. The  $\text{K}^+$  current is  $\text{Ca}^{2+}$ -dependent, voltage-independent, and completely blocked by the  $\text{K}^+$  channel blockers charybdotoxin, margatoxin, and iberiotoxin with IC50 values of 1.7, 16, and 62 nM, resp. The underlying  $\text{K}^+$  channels possess a single channel conductance of 33 pS in sym.  $\text{K}^+$  soln. Pretreatment of cells with pertussis toxin (PTX), *Clostridium sordellii* lethal toxin, or a farnesyl protein transferase inhibitor reduced the  $\text{K}^+$  current amplitude in response to LPA to about 25% of the control value. Incubation of cells with the protein tyrosine kinase inhibitor genistein or microinjection of the neutralizing anti-**Ras** monoclonal antibody Y13-259 reduced it by more than 50%. In contrast, the phospholipase C inhibitor U-73122 and the protein kinase A activator 8-bromo-cAMP had no effect. These results indicate that the  $\text{K}^+$  channel activation by LPA is mediated by a signal transduction pathway involving a PTX-sensitive G protein, a protein tyrosine kinase, and **Ras**. LPA is already known to activate  $\text{Cl}^-$  channels in various cell types, thereby leading to membrane depolarization. In conjunction

Searcher : Shears 308-4994

09/126816

with our results that demonstrate LPA-induced membrane hyperpolarization by activation of K<sup>+</sup> channels, LPA appears to be significantly involved in the regulation of the cellular membrane potential.

L5 ANSWER 8 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1998:524147 CAPLUS  
DOCUMENT NUMBER: 129:227404  
TITLE: A common motif of eukaryotic glycosyltransferases is essential for the enzyme activity of large clostridial cytotoxins  
AUTHOR(S): Busch, Christian; Hofmann, Fred; Selzer, Jorg; Munro, Sean; Jeckel, Dieter; Aktories, Klaus  
CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Freiburg, D-79104, Germany  
SOURCE: J. Biol. Chem. (1998), 273(31), 19566-19572  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB A fragment of the N-terminal 546 amino acid residues of *Clostridium sordellii* lethal toxin possesses full enzyme activity and glucosylates Rho and Ras GTPases in vitro. Here we identified several amino acid residues in C. *sordellii* lethal toxin that are essential for the enzyme activity of the active toxin fragment. Exchange of aspartic acid at position 286 or 288 with alanine or asparagine decreased glucosyltransferase activity by about 5000-fold and completely blocked glucohydrolase activity. No enzyme activity was detected with the double mutant D286A/D288A. Whereas the wild-type fragment of C. *sordellii* lethal toxin was labeled by azido-UDP-glucose after UV irradn., mutation of the DXD motif prevented radiolabeling. At high concns. (10 mM) of manganese ions, the transferase activities of the D286A and D288A mutants but not that of wild-type fragment were increased by about 20-fold. The exchange of Asp270 and Arg273 reduced glucosyltransferase activity by about 200-fold and blocked glucohydrolase activity. The data indicate that the DXD motif, which is highly conserved in all large clostridial cytotoxins and also in a large no. of glycosyltransferases, is functionally essential for the enzyme activity of the toxins and may participate in coordination of the divalent cation and/or in the binding of UDP-glucose.

L5 ANSWER 9 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1998:425281 CAPLUS  
DOCUMENT NUMBER: 129:158435  
Searcher : Shears 308-4994

09/126816

TITLE: Functional consequences of monoglucosylation of Ha-Ras at effector domain amino acid threonine 35

AUTHOR(S): Herrmann, Christian; Reza, Mohammad Reza; Hofmann, Fred; Just, Ingo

CORPORATE SOURCE: Inst. Pharmakol. Toxikol., Univ. Friburg, Hermann-Herder-Strasse, D-79104, Germany

SOURCE: J. Biol. Chem. (1998), 273(26), 16134-16139

CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Monoglucosylation of low mol. mass GTPases is an important post-translational modification by which microbes interfere with eukaryotic cell signaling. Ha-Ras is monoglucosylated at effector domain amino acid threonine 35 by Clostridium sordellii lethal toxin, resulting in a blockade of the downstream mitogen-activated protein kinase cascade. To understand the mol. consequences of this modification, effects of glucosylation on each step of the GTPase cycle of Ras were analyzed. Whereas nucleotide binding was not significantly altered, intrinsic GTPase activity was markedly decreased, and GTPase stimulation by the GTPase-activating protein p120GAP and neurofibromin NF-1 was completely blocked, caused by failure to bind to glucosylated Ras. Guanine nucleotide exchange factor (Cdc25)-catalyzed GTP loading was decreased, but not completely inhibited. A dominant-neg. property of modified Ras to sequester exchange factor was not detectable. However, the crucial step in downstream signaling, Ras-effector coupling, was completely blocked. The Kd for the interaction between Ras-GTP and the Ras-binding domain of Raf was 15 nM, whereas glucosylation increased the Kd to >1 mM. Because the affinity of Ras.cntdot.GDP for Raf (Kd = 22 .mu.M) is too low to allow functional interaction, a glucose moiety at threonine 35 of Ras seems to block completely the interaction with Raf. The net effect of lethal toxin-catalyzed glucosylation of Ras is the complete blockade of Ras downstream signaling.

L5 ANSWER 10 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:271846 CAPLUS

DOCUMENT NUMBER: 129:52440

TITLE: Rho protein inhibition blocks protein kinase C translocation and activation

AUTHOR(S): Hippenstiel, Stefan; Kratz, Thomas; Krull, Matthias; Seybold, Joachim; Eichel-Streiber, Christoph V.; Suttorp, Norbert

CORPORATE SOURCE: Department of Internal Medicine,  
Searcher : Shears 308-4994

09/126816

Justus-Liebig-University, Giessen, D-35392,  
Germany  
SOURCE: Biochem. Biophys. Res. Commun. (1998), 245(3),  
830-834  
CODEN: BBRCA9; ISSN: 0006-291X  
PUBLISHER: Academic Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Small GTP-binding proteins of the **Ras** and Rho family participate in various important signalling pathways. Large clostridial cytotoxins inactivate GTPases by UDP-glucosylation. Using *Clostridium difficile* toxin B-10463 (TcdB) for inactivation of Rho proteins (RhoA/Rac/Cdc42) and *Clostridium sordellii* **lethal toxin**-1522 (TcsL) for inactivation of **Ras**-proteins (**Ras**/Rac/Ral, Rap) the role of these GTPases in protein kinase C (PKC) stimulation was studied. Phorbol-myristate-acetate (PMA) induced a rapid PKC translocation to and activation in the particulate cell fraction as detd. by PKC-activity measurements and Western blots for PKC. $\alpha$ . These effects were blocked by TcdB inhibiting Rho proteins in endothelial cells, but not in TcsL-treated cells (i.e., cells without **Ras** activity), suggesting that Rho GTPases (RhoA and/or Cdc42) are the most likely GTP-binding proteins responsible for PKC activation. The Rho requirement for PKC activation/translocation was also verified for human epithelial cells and for lipopolysaccharide-stimulated endothelial cells. In summary, the data presented indicate that Rho protein inhibition blocked PKC translocation/activation in endothelial and epithelial cells.

L5 ANSWER 11 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1998:223590 CAPLUS  
DOCUMENT NUMBER: 128:318211  
TITLE: Specific inhibition of phorbol ester-stimulated phospholipase D by *Clostridium sordellii* **lethal toxin** and *Clostridium difficile* toxin B-1470 in HEK-293 cells. Restoration by Ral GTPases  
AUTHOR(S): Schmidt, Martina; Voss, Matthias; Thiel, Markus; Bauer, Bettina; Grannass, Andreas; Tapp, Eva; Cool, Robbert H.; De Gunzburg, Jean; Von Eichel-Streiber, Christoph; Jakobs, Karl H.  
CORPORATE SOURCE: Universitätsklinikum Essen, Institut für Pharmakologie, Essen, D-45122, Germany  
SOURCE: J. Biol. Chem. (1998), 273(13), 7413-7422  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

Searcher : Shears 308-4994

09/126816

AB To study whether **Ras**-like GTPases are involved in phospholipase D (PLD) regulation, we studied the effects of the *Clostridium difficile* toxin B (TcdB) variant TcdB-1470 and *Clostridium sordellii* lethal toxin (TcsL), known to inactivate Rac and some members of the **Ras** protein family, on PLD activities. TcdB-1470 and TcsL did not affect basal PLD activity and PLD stimulation by m<sub>3</sub> muscarinic acetylcholine receptor (mAChR) or direct G protein activation. In contrast, PMA-induced PLD stimulation was inhibited by TcdB-1470 and TcsL in a time-and concn.-dependent manner, without alteration in immunol. detectable protein kinase C (PKC) isoenzyme levels. In membranes of HEK-293 cells pretreated with TcdB-1470 or TcsL, basal and stable GTP analog-stimulated PLD activities measured with exogenous phosphatidylcholine, in the presence or absence of phosphatidylinositol 4,5-bisphosphate, were not altered. In contrast, pretreatment with TcdB-1470 and TcsL, but not TcdB, strongly reduced PMA-stimulated PLD activity. The addn. of recombinant Rac1, serving as glucosylation substrate for TcdB, TcsL, and TcdB-1470, did not restore PLD stimulation by PMA. Furthermore, PMA-stimulated PLD activity, suppressed by prior treatment with TcdB-1470 or TcsL, was not rescued by the addn. of recombinant **Ras** (RasG12V) or Rap proteins, acting as glucosylation substrates for TcsL only (**Ras**) or TcdB-1470 and TcsL (Rap). In contrast, the addn. of recombinant Ral proteins (RalA and RalB), glucosylation substrates for TcsL and TcdB-1470, but not for TcdB, to membranes of TcdB-1470- or TcsL-treated cells fully restored PLD stimulation by PMA without altering the strict MgATP dependence of PMA-induced PLD stimulation. RalA-mediated restoration of PMA-stimulated PLD activity in membranes of TcsL-treated cells was not enhanced by coaddn. of RasG12V. In conclusion, the data presented indicate that TcdB-1470 and TcsL selectively interfere with phorbol ester stimulation of PLD and suggest an essential role of Ral proteins in PKC signaling to PLD in HEK-293 cells.

L5 ANSWER 12 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1998:207001 CAPLUS  
DOCUMENT NUMBER: 129:14892  
TITLE: Activation of a Ca<sup>2+</sup>-dependent K<sup>+</sup> current by the oncogenic receptor protein tyrosine kinase v-Fms in mouse fibroblasts  
AUTHOR(S): Decker, Katrin; Koschinski, Andreas; Trouliaris, Sylvia; Tamura, T.; Dreyer, Florian; Repp, H.  
CORPORATE SOURCE: Rudolf-Buchheim-Institut fur Pharmakologie, Justus-Liebig-Universitat Giessen, Frankfurter Strasse 107, Giessen, D-35392, Germany  
SOURCE: Naunyn-Schmiedeberg's Arch. Pharmacol. (1998), 357(4), 378-384  
CODEN: NSAPCC; ISSN: 0028-1298  
Searcher : Shears 308-4994

09/126816

PUBLISHER: Springer-Verlag  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB We investigated the effects of the receptor-coupled protein tyrosine kinase (RTK) v-Fms on the membrane current properties of NIH3T3 mouse fibroblasts. We found that v-Fms, the oncogenic variant of the macrophage colony-stimulating factor receptor c-Fms, activates a K<sup>+</sup> current that is absent in control cells. The activation of the K<sup>+</sup> current was Ca<sup>2+</sup>-dependent, voltage-independent, and was completely blocked by the K<sup>+</sup> channel blockers charybdotoxin, margatoxin and iberiotoxin with IC<sub>50</sub> values of 3 nM, 18 nM and 76 nM, resp. To identify signaling components that mediate the activation of this K<sup>+</sup> current, NIH3T3 cells that express different mutants of the wildtype v-Fms receptor were examined. Mutation of the binding site for the Ras-GTPase-activating protein led to a complete abolishment of the K<sup>+</sup> current. A redn. of 76% and 63%, resp., was obsd. upon mutation of either of the two binding sites for the growth factor receptor binding protein 2. Mutation of the ATP binding lobe, which disrupts the protein tyrosine kinase activity of v-Fms, led to a 55% redn. of the K<sup>+</sup> current. Treatment of wild-type v-Fms cells with *Clostridium sordellii* **lethal toxin** or a farnesyl protein transferase inhibitor, both known to inhibit the biol. function of **Ras**, reduced the K<sup>+</sup> current amplitude to 17% and 6% of the control value, resp. This is the first report showing that an oncogenic RTK can modulate K<sup>+</sup> channel activity. Our results indicate that this effect is dependent on the binding of certain **Ras**-regulating proteins to the v-Fms receptor and is not abolished by disruption of its intrinsic protein tyrosine kinase activity. Furthermore, our data suggest that **Ras** plays a key role for K<sup>+</sup> channel activation by the oncogenic RTK v-Fms.

L5 ANSWER 13 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1998:130788 CAPLUS  
DOCUMENT NUMBER: 128:253966  
TITLE: Chimeric clostridial cytotoxins: identification of the N-terminal region involved in protein substrate recognition  
AUTHOR(S): Hofmann, Fred; Busch, Christian; Aktories, Klaus  
CORPORATE SOURCE: Institute fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Freiburg, D-79104, Germany  
SOURCE: Infect. Immun. (1998), 66(3), 1076-1081  
CODEN: INFIBR; ISSN: 0019-9567  
PUBLISHER: American Society for Microbiology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB **Clostridium sordellii** **lethal toxin** is a member of the family of large clostridial cytotoxins that  
Searcher : Shears 308-4994

09/126816

glucosylate small GTPases. In contrast to *Clostridium difficile* toxins A and B, which exclusively modify Rho subfamily proteins, *C. sordellii* lethal toxin also glucosylates Ras subfamily proteins. By deletion anal. and construction of chimeric fusion proteins of *C. sordellii* lethal toxin and *C. difficile* toxin B, we localized the enzyme activity of the lethal toxin to the N terminus of the holotoxin and identified the region involved in protein substrate specificity. The toxin fragment of the N-terminal 546 amino acid residues of *C. sordellii* lethal toxin glucosylated Rho and Ras subfamily proteins, as the holotoxin did. Deletion of a further 30 amino acid residues from the C terminus of this active fragment drastically reduced glucotransferase activity and blocked glucohydrolase activity. Exchange of amino acid residues 364 through 516 of lethal toxin for those in the active toxin B fragment (1 to 546) allowed glucosylation of Ras subfamily proteins. In contrast, the chimera with amino acids 1 to 364 from toxin B, 365 to 468 from lethal toxin, and 469 to 546 from toxin B exhibited markedly reduced modification of Ras subfamily proteins, whereas modification of Rac and Cdc42 was hardly changed. The data indicate that the region of amino acid residues 364 through 516 primarily defines the substrate specificity of *C. sordellii* lethal toxin.

L5 ANSWER 14 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1997:713569 CAPLUS  
DOCUMENT NUMBER: 128:10495  
TITLE: Evidence for differential roles of the Rho subfamily of GTP-binding proteins in glucose- and calcium-induced insulin secretion from pancreatic .beta. cells  
AUTHOR(S): Kowluru, Anjaneyulu; Li, Guodong; Rabaglia, Mary E.; Segu, Venkatesh B.; Hofmann, Fred; Aktories, Klaus; Metz, Stewart A.  
CORPORATE SOURCE: WILLIAM S. MIDDLETON MEMORIAL VA MEDICAL CENTER, MEDICAL AND RESEARCH SERVICES, MADISON, WI, 53705, USA  
SOURCE: Biochem. Pharmacol. (1997), 54(10), 1097-1108  
CODEN: BCPCA6; ISSN: 0006-2952  
PUBLISHER: Elsevier  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB We utilized clostridial toxins (with known specificities for inhibition of GTPases) to ascertain the contribution of candidate GTPases in physiol. insulin secretion from .beta. cells. Exposure of normal rat islets or isolated .beta. (HIT-T15) cells to *Clostridium difficile* toxins A and B catalyzed the glucosylation (and thereby the inactivation) of Rac, Cdc42, and Rho endogenous to  
Searcher : Shears 308-4994

09/126816

.beta. cells; concomitantly, either toxin reduced glucose- or potassium-induced insulin secretion from rat islets and HIT cells. Treatment of .beta. cells with **Clostridium sordellii** **lethal toxin** (LT; which modified only **Ras**, **Rap**, and **Rac**) also reduced glucose- or potassium-induced secretion. However, clostridial toxin C3-exoenzyme (which ADP-ribosylates and inactivates only Rho) was without any effect on either glucose- or potassium-induced insulin secretion. These data suggest that Cdc42, Rac, **Ras**, and/or Rap (but not Rho) may be needed for glucose- or potassium-mediated secretion. The effects of these **toxins** appear to be specific on stimulus-secretion coupling, since no difference in metabolic viability (assessed colorimetrically by quantitating the conversion of the tetrazolium salt into a formazan in a redn. reaction driven by nutrient metab.) was demonstrable between control and **toxin** (A or LT)-treated .beta. cells. **Toxin** (A or LT) treatment also did not alter glucose- or potassium-mediated rises in cytosolic free calcium concns. ([Ca<sup>2+</sup>]<sub>i</sub>), suggesting that these GTPases are involved in steps distal to elevations in [Ca<sup>2+</sup>]<sub>i</sub>. Recent findings indicate that the carboxyl methylation of Cdc42 is stimulated by only glucose, whereas that of Rap (A. Kowluru et al., 1996) and Rac (present study) are regulated by glucose or potassium. Together, these findings provide direct evidence, for the first time, that the Rho subfamily of GTPases plays a key regulatory role(s) in insulin secretion, and they suggest that Cdc42 may be required for early steps in glucose stimulation of insulin release, whereas Rap and/or Rac may be required for a later step(s) in the stimulus-secretion coupling cascade (i.e. Ca<sup>2+</sup>-induced exocytosis of insulin).

L5 ANSWER 15 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1997:533546 CAPLUS  
DOCUMENT NUMBER: 127:195467  
TITLE: Immunotoxin inactivation of **Ras**  
subfamily proteins and agents therefor  
INVENTOR(S): Von Eichel-Streiber, Christoph; Boquet, Patrice;  
Thelestam, Monica  
PATENT ASSIGNEE(S): Boehringer Mannheim G.m.b.H., Germany; Von  
Eichel-Streiber, Christoph; Boquet, Patrice;  
Thelestam, Monica  
SOURCE: PCT Int. Appl., 45 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

| PATENT NO. | KIND     | DATE  | APPLICATION NO. | DATE     |
|------------|----------|-------|-----------------|----------|
| -----      | -----    | ----- | -----           | -----    |
|            | Searcher | :     | Shears          | 308-4994 |

09/126816

WO 9727871 A1 19970807 WO 1997-EP426 19970131  
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ,  
DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR,  
KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO,  
NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA,  
UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB,  
GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA,  
GN, ML, MR, NE, SN, TD, TG  
AU 9715982 A1 19970822 AU 1997-15982 19970131  
EP 877622 A1 19981118 EP 1997-902278 19970131  
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC,  
PT, IE, FI  
PRIORITY APPLN. INFO.: EP 1996-101469 19960202  
WO 1997-EP426 19970131

AB The invention comprises a method of treating a patient with a disorder, characterized by an activating mutation in the **Ras** proto-oncogene, comprising contacting cells of said patient with a protein having the toxic activity of **Clostridium sordellii** toxin **LT** under conditions favoring inactivating of **Ras** by glucosylation of **Ras**' threonine 35 in said cell. Said protein preferably is an immunotoxin which contains as a toxic domain the catalytic domain of **toxin LT**.

L5 ANSWER 16 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1997:510330 CAPLUS  
DOCUMENT NUMBER: 127:172444  
TITLE: Escherichia coli cytotoxic necrotizing factor 1 (CNF1), a toxin that activates the Rho GTPase  
AUTHOR(S): Fiorentini, Carla; Fabbri, Alessia; Flatau, Gilles; Donelli, Gianfranco; Matarrese, Paola; Lemichez, Emmanuel; Falzano, Loredana; Boquet, Patrice  
CORPORATE SOURCE: Dep. Ultrastructures, Inst. Superiore Sanita, Rome, 00161, Italy  
SOURCE: J. Biol. Chem. (1997), 272(31), 19532-19537  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Cytotoxic necrotizing factor 1 (CNF1), a 110-kDa protein toxin from pathogenic Escherichia coli induces actin reorganization into stress fibers and retraction fibers in human epithelial cultured cells allowing them to spread. CNF1 is acting in the cytosol since microinjection of the toxin into HEp-2 cells mimics the effects of the externally applied CNF1. Incubation in vitro of CNF1 with recombinant small GTPases induces a modification of Rho (but not of

Searcher : Shears 308-4994

09/126816

Rac, Cdc42, Ras, or Rab6) as demonstrated by a discrete increase in the apparent mol. wt. of the mol. Preincubation of cells with CNF1 impairs the cytotoxic effects of Clostridium difficult toxin B, which inactivates Rho but not those of Clostridium sordellii LT toxin, which inhibits Ras and Rac. As shown for Rho-GTP, CNF1 activates, in a time- and dose-dependent manner, a cytoskeleton-assocd. phosphatidylinositol 4-phosphate 5-kinase. However, neither the phosphatidylinositol 4,5-bisphosphate (PI 3,4-P2) or 3,4,5-trisphosphate (PIP3) cellular content were found increased in CNF1 treated HEp-2 cells. Cellular effects of CNF1 were not blocked by LY294002, a stable inhibitor of the phosphoinositide 3-kinase. Incubation of HEp-2 cells with CNF1 induces relocalization of myosin 2 in stress fibers but not in retraction fibers. Altogether, our data indicate that CNF1 is a toxin that selectively activates the Rho GTP-binding protein, thus inducing contractility and cell spreading.

L5 ANSWER 17 OF 20 CAPLUS COPYRIGHT 1999 ACS .

ACCESSION NUMBER: 1996:761992 CAPLUS  
DOCUMENT NUMBER: 126:43823  
TITLE: Difference in protein substrate specificity  
between hemorrhagic toxin and lethal  
toxin from Clostridium sordellii  
AUTHOR(S): Genth, Harald; Hofmann, Fred; Selzer, Joerg;  
Aktories, Klaus; Just, Ingo  
CORPORATE SOURCE: Institut fuer Pharmakologie der  
Albert-Ludwigs-Universitaet Freiburg, Freiburg,  
D-79104, Germany  
SOURCE: Biochem. Biophys. Res. Commun. (1996), 229(2),  
370-374  
CODEN: BBRCA9; ISSN: 0006-291X  
PUBLISHER: Academic  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Here we report that hemorrhagic toxin (HT), which is coexpressed with lethal toxin, is also a glucosyltransferase. Whereas lethal toxin glycosylates the Rho subfamily proteins Rac and Cdc42 and the Ras subfamily proteins H-Ras and Rap, the substrate specificity of HT is strictly confined to the Rho subfamily proteins Rho, Rac and Cdc42. Comparable to lethal toxin, transferase activity of HT is stimulated by Mn<sup>2+</sup>. Acceptor amino acid in Rho was identified by mutagenesis as threonine-37. C. sordellii HT is a novel member of the family of clostridial mono-glucosyl-transferases, a family which modifies the Rho and Ras of GTPases.

L5 ANSWER 18 OF 20 CAPLUS COPYRIGHT 1999 ACS

Searcher : Shears 308-4994

09/126816

ACCESSION NUMBER: 1996:606610 CAPLUS  
DOCUMENT NUMBER: 125:240634  
TITLE: The Ras-related protein Ral is  
monoglycosylated by Clostridium  
**sordellii** lethal toxin  
AUTHOR(S): Hofmann, Fred; Rex, Gundula; Aktories, Klaus;  
Just, Ingo  
CORPORATE SOURCE: Institut fuer Pharmakologie und Toxikologie,  
AlbertLudwigs-Universitaet Freiburg, Freiburg,  
D-79104, Germany  
SOURCE: Biochem. Biophys. Res. Commun. (1996), 227(1),  
77-81  
CODEN: BBRCA9; ISSN: 0006-291X  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB We report here on **lethal toxin (LT)**  
produced by **C. sordellii** strain 6018 which glucosylates in  
addn. to Rac, Ras and Rap the Ral protein. LT from strain  
VPI19048 however does not glucosylate Ral. Besides recombinant Ral,  
cellular Ral is also substrate. In the GDP-bound form, Ral is a  
superior substrate to the GTP form. Acceptor amino acid for glucose  
is threonine-46 which is equiv. to threonine-35 in H-Ras  
located in the effector region. The Ral-glucosylating toxin is a  
novel isoform of **Ras**-modifying clostridial cytotoxins.

L5 ANSWER 19 OF 20 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1996:256012 CAPLUS  
DOCUMENT NUMBER: 124:309937  
TITLE: Ras, Rap, and Rac small GTP-binding  
proteins are targets for Clostridium  
**sordellii** lethal toxin  
glucosylation  
AUTHOR(S): Popoff, Michel R.; Chaves-Olarde, Esteban;  
Lemichez, Emmanuel; von Eichel-Streiber,  
Christoph; Thelestam, Monica; Chardin, Pierre;  
Cussac, Didier; Antonny, Bruno; Chavrier,  
Philippe; et al.  
CORPORATE SOURCE: Inst. Pasteur, Unite Toxines Microbiennes,  
Paris, 75724, Fr.  
SOURCE: J. Biol. Chem. (1996), 271(17), 10217-24  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB **Lethal toxin (LT)** from Clostridium  
**sordellii** is one of the high mol. mass clostridial  
cytotoxins. On cultured cells, it causes a rounding of cell bodies  
and a disruption of actin stress fibers. We demonstrate that LT is  
a glucosyltransferase that uses UDP-Glc as a cofactor to covalently  
modify 21-kDa proteins both in vitro and in vivo. LT glucosylates  
Searcher : Shears 308-4994

09/126816

Ras, Rap, and Rac. In Ras, threonine at position 35 was identified as the target amino acid glucosylated by LT. Other related members of the Ras GTPase superfamily, including RhoA, Cdc42, and Rab6, were not modified by LT. Incubation of serum-starved Swiss 3T3 cells with LT prevents the epidermal growth factor-induced phosphorylation of mitogen-activated protein kinases ERK1 and ERK2, indicating that the toxin blocks Ras function in vivo. We also demonstrate that LT acts inside the cell and that the glucosylation reaction is required to observe its dramatic effect on cell morphol. LT is thus a powerful tool to inhibit Ras function in vivo.

L5 ANSWER 20 OF 20 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1996:256001 CAPLUS  
DOCUMENT NUMBER: 124:309936  
TITLE: Inactivation of Ras by Clostridium  
sordellii lethal toxin  
-catalyzed glucosylation  
AUTHOR(S): Just, Ingo; Selzer, Joerg; Hofmann, Fred; Green,  
Gaynor A.; Aktories, Klaus  
CORPORATE SOURCE: Inst. Pharmakol. Toxikol., Univ. Freiburg,  
Freiburg, D-79104, Germany  
SOURCE: J. Biol. Chem. (1996), 271(17), 10149-53  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The lethal toxin (LT) from Clostridium sordellii belongs to the family of large clostridial cytotoxins causing morphol. alterations in cultured cell lines accompanied by destruction of the actin cytoskeleton. C.. sordellii LT exhibits 90% homol. to Clostridium difficile toxin B, which has been recently identified as a monoglucosyltransferase (1995). We report here that LT too is a glucosyltransferase, which uses UDP-glucose as cosubstrate to modify low mol. mass GTPases. LT selectively modified Rac and Ras, whereas the substrate specificity of toxin B is confined to the Rho subfamily proteins Rho, Rac, and Cdc42, which participate in the regulation of the actin cytoskeleton. In Rac, both toxin B and LT share the same acceptor amino acid, threonine 35. Glucosylation of Ras by LT results in inhibition of the epidermal growth factor-stimulated p42/p44 MAP-kinase signal pathway. LT is the first bacterial toxin to inactivate Ras in intact cells.

(FILE 'MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, PROMT, TOXLIT, TOXLINE, CANCERLIT' ENTERED AT 11:35:20 ON 16 NOV 1999)

Searcher : Shears 308-4994

09/126816

L6 124 S L5  
L7 32 DUP REM L6 (92 DUPLICATES REMOVED)

L7 ANSWER 1 OF 32 TOXLINE  
ACCESSION NUMBER: 1999:73084 TOXLINE  
DOCUMENT NUMBER: FEDRIP-1999-06409240  
TITLE: GTP Binding Proteins in Islet Signal Transduction.  
AUTHOR: Kowluru A  
CORPORATE SOURCE: Department of Veterans Affairs/Medical Center,  
Madison, WI  
Department of Veterans Affairs/Research and  
Development (15), 810 Vermont Ave. N.W., Washington,  
D.C.  
CONTRACT NUMBER: VA 00220657  
SOURCE: (1999). FEDRIP DATABASE, NATIONAL TECHNICAL  
INFORMATION SERVICE (NTIS).  
FILE SEGMENT: FEDRIP  
LANGUAGE: Unavailable  
ENTRY MONTH: 199904  
AB RPROJ/FEDRIP SIGNAL TRANSDUCTION; PHOSPHOLIPASES; MOLECULAR WEIGHT;  
G-PROTEINS; EXOCYTOSIS OBJECTIVE: To examine the role of Rho  
subfamily GTPases in nutrient and calcium-mediated insulin secretion.  
RESEARCH PLAN AND METHODOLOGY: We utilized Clostridial  
toxins (with known specificities for inhibition of GTPases)  
to ascertain the contribution of candidate GTPases in physiologic  
insulin secretion from pancreatic beta cells. FINDINGS: Exposure of  
normal rat islets or isolated beta (HIT-T15) cells to Clostridium  
difficile toxins A or B catalyzed the glycosylation (and  
thereby inactivation) of Rac, Cdc42, and Rho endogenous to beta  
cells; concomitantly, either toxin reduced glucose- or  
potassium-induced insulin secretion from rat islets and HIT cells.  
Treatment of beta cells with Clostridium sordellii  
lethal toxin (LT; which modified only  
Ras, Rap and Rac) also reduced glucose or potassium-induced  
secretion. However, Clostridial toxin C3-exoenzyme (which  
ADP-ribosylates and inactivates only Rho) was without any effect on  
either glucose or potassium-induced insulin secretion. These data  
suggest that Cdc42, Rac, Ras and/or Rap (but not Rho) may  
be needed for glucose or potassium-mediated secretion. Effects of  
these toxins appear to be specific on stimulus-secretion  
coupling, since no difference in metabolic viability (assessed  
colorimetrically by quantitating the conversion of tetrazolium salt  
into a formazan in a reduction reaction driven by nutrient  
metabolism) was demonstrable between control and toxin (A  
or LT)-treated beta cells. Toxin (A or  
LT)-treatment also did not alter glucose or  
potassium-mediated rises in cytosolic free calcium concentrations,  
suggesting that these GTPases are involved in steps distal to  
elevation in cytosolic calcium. Recent findings indicate that the

Searcher : Shears 308-4994

09/126816

carboxyl methylation of Cdc42 is stimulated by only glucose, whereas that of Rap and Rac (present study) are regulated by glucose or potassium. SIGNIFICANCE: These provide direct evidence, for the first time, that Rho subfamily of GTPase play key regulatory role(s) in insulin secretion and suggest that Cdc42 may be required for early steps in glucose stimulation of insulin release, whereas and Rap and/or Rac may be required for a early steps(s) in the stimulus-secretion coupling cascade. These data provide insights into the roles of Rho subfamily GTPases in physiologic insulin secretion which form the basis for our future studies on the metabolic regulation of these proteins in animal models of impaired insulin secretion. Data imply that the CM of gamma subunits in insulin-secreting cells may be facilitated by dissociation of the alpha/beta/gamma trimer into alpha and beta/gamma. Regulation of such a cascade by glucose, an effect dependent on calcium influx and the consequent activation of phospholipases releasing arachidonic acid, implies an important role of the CM of gamma subunits in beta cell function.

L7 ANSWER 2 OF 32 TOXLIT

ACCESSION NUMBER: 1999:68792 TOXLIT

DOCUMENT NUMBER: CA-131-209119P

TITLE: Toxicologically active fragments of lethal  
toxin from *Clostridium sordellii*  
and their application in immunotoxins.

AUTHOR: Aktories K; Hofmann F

SOURCE: (1999). Ger. Offen. PATENT NO. 19802569 09/09/1999  
(Albert-Ludwigs-Universitaet Freiburg).

CODEN: GWXXBX.

PUB. COUNTRY: GERMANY, FEDERAL REPUBLIC OF

DOCUMENT TYPE: Patent

FILE SEGMENT: CA

LANGUAGE: German

OTHER SOURCE: CA 131:209119

ENTRY MONTH: 199910

AB Fragment 1-546 of *C. sordellii* lethal  
toxin and an immunotoxin comprising this protein fused to a  
cell-binding moiety, such as a tumor cell-binding antibody or  
antibody fragment, are disclosed. The immunotoxin may addnl. contain  
a translocation signal, e.g., the translocation domain of  
*Pseudomonas* exotoxin A or of the *Clostridium* C2 toxin. The 1-546  
fragment of the *C. sordellii* lethal  
toxin was found to have higher glucosyltransferase activity  
with Ras as substrate than did the wild-type  
lethal toxin.

L7 ANSWER 3 OF 32 MEDLINE

ACCESSION NUMBER: 1999253957 MEDLINE

DOCUMENT NUMBER: 99253957

DUPPLICATE 1

Searcher : Shears 308-4994

09/126816

TITLE: G-protein-stimulated phospholipase D activity is inhibited by **lethal toxin** from **Clostridium sordellii** in HL-60 cells.

AUTHOR: El Hadj N B; Popoff M R; Marvaud J C; Payrastre B; Boquet P; Geny B

CORPORATE SOURCE: INSERM U332, ICGM, 22 rue Mecham, 75014 Paris, France.

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 May 14) 274 (20) 14021-31.

Journal code: HIV. ISSN: 0021-9258.

PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199908

ENTRY WEEK: 19990803

AB **Lethal toxin (LT) from Clostridium sordellii** has been shown in HeLa cells to glucosylate and inactivate **Ras** and **Rac** and, hence, to disorganize the actin cytoskeleton. In the present work, we demonstrate that **LT** treatment provokes the same effects in HL-60 cells. We show that guanosine 5'-O-(3-thiotriphosphate)-stimulated phospholipase D (PLD) activity is inhibited in a time- and dose-dependent manner after an overnight treatment with **LT**. A similar dose response to the **toxin** was found when PLD activity was stimulated by phorbol 12-myristate 13-acetate via the protein kinase C pathway. The **toxin** effect on actin organization seemed unlikely to account directly for PLD inhibition as cytochalasin D and iota **toxin** from **Clostridium perfringens** E disorganize the actin cytoskeleton without modifying PLD activity. However, the enzyme inhibition and actin cytoskeleton disorganization could both be related to a major decrease observed in phosphatidylinositol 4,5-bisphosphate (PtdIns(4, 5)P2). Likely in a relationship with this decrease, recombinant ADP-ribosylation factor, RhoA, Rac, and RalA were not able to reconstitute PLD activity in **LT**-treated cells permeabilized and depleted of cytosol. Studies of phosphoinositide kinase activities did not allow us to attribute the decrease in PtdIns(4, 5)P2 to inactivation of PtdIns4P 5-kinase. **LT** was also found to provoke a major inhibition in phosphatidylinositol 3-kinase that could not account for the inhibition of PLD activity because wortmannin, at doses that fully inhibit phosphatidylinositol 3-kinase, had no effect on the phospholipase activity. Among the three small G-proteins, **Ras**, **Rac**, and **RalA**, inactivated by **LT** and involved in PLD regulation, inactivation of **Ral** proteins appeared to be responsible for PLD inhibition as **LT toxin** (strain 9048) unable to glucosylate **Ral** proteins did not modify PLD activity. In HL-60 cells, **LT** treatment appeared also to modify cytosol components in relationship with PLD inhibition as a

Searcher : Shears 308-4994

09/126816

cytosol prepared from LT-treated cells was less efficient than one from control HL-60 cells in stimulating PLD activity. Phosphatidylinositol transfer proteins involved in the regulation of polyphosphoinositides and ADP-ribosylation factor, a major cytosolic PLD activator in HL-60 cells, were unchanged, whereas the level of cytosolic protein kinase Calpha was decreased after LT treatment. We conclude that in HL-60 cells, lethal toxin from *C. sordellii*, in inactivating small G-proteins involved in PLD regulation, provokes major modifications at the membrane and the cytosol levels that participate in the inhibition of PLD activity. Although Ral appeared to play an essential role in PLD activity, we discuss the role of other small G-proteins inactivated by LT in the different modifications observed in HL-60 cells.

L7 ANSWER 4 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)

ACCESSION NUMBER: 1999:307363 SCISEARCH

THE GENUINE ARTICLE: 186VV

TITLE: A novel cytotoxin from *Clostridium difficile* serogroup F is a functional hybrid between two other large clostridial cytotoxins

AUTHOR: ChavesOlarte E; Low P; Freer E; Norlin T; Weidmann M; vonEichelStreiber C; Thelestam M (Reprint)

CORPORATE SOURCE: KAROLINSKA INST, CTR MICROBIOL & TUMOR BIOL, BOX 280, S-17177 STOCKHOLM, SWEDEN (Reprint); KAROLINSKA INST, CTR MICROBIOL & TUMOR BIOL, S-17177 STOCKHOLM, SWEDEN; UNIV COSTA RICA, UNIDAD MICROSCOPIA ELECT, SAN JOSE, COSTA RICA; KAROLINSKA INST, NOBEL INST NEUROPHYSIOL, DEPT NEUROSCI, S-17177 STOCKHOLM, SWEDEN; UNIV MAINZ, INST MED MIKROBIOL & HYG, VERFUGUNGEBAUDE FORSCH & ENTWICKLUNG, D-55101 MAINZ, GERMANY

COUNTRY OF AUTHOR: SWEDEN; COSTA RICA; GERMANY

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (16 APR 1999) Vol. 274, No. 16, pp. 11046-11052.

Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.

ISSN: 0021-9258.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 21

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The large clostridial cytotoxins (LCTs) constitute a group of high molecular weight clostridial cytotoxins that inactivate cellular small GTP-binding proteins. We demonstrate that a novel LCT (TcdB-1470) from *Clostridium difficile* strain 1470 is a functional hybrid between 'reference' TcdB-10463 and *Clostridium sordellii* TcsL-1522. It bound to the same specific receptor

Searcher : Shears 308-4994

09/126816

as TcdB-10463 but glucosylated the same GTP-binding proteins as TcsL-1522. All three toxins had equal enzymatic potencies but were equally cytotoxic only when micro injected. When applied extracellularly TcdB-1470 and TcdB-10463 were considerably more potent cytotoxins than TcsL-1522. The small GTP-binding protein R-Ras was identified as a target for TcdB-1470 and also for Test-1522 but not for TcdB-10463. R-Ras is known to control integrin-extracellular matrix interactions from inside the cell. Its glucosylation may be a major determinant for the cell rounding and detachment induced by the two R-Ras-attacking toxins. In contrast, fibroblasts treated with TcdB-10463 were arborized and remained attached, with phosphotyrosine containing structures located at the cell-to-cell contacts and beta(3)-integrin remaining at the tips of cellular protrusions. These components were absent from cells treated with the R-Ras-inactivating toxins. The novel hybrid toxin will broaden the utility of the LCTs for clarifying the functions of several small GTPases, now including also R-Ras.

L7 ANSWER 5 OF 32 MEDLINE DUPLICATE 2  
ACCESSION NUMBER: 1999270964 MEDLINE  
DOCUMENT NUMBER: 99270964  
TITLE: Effects of cytotoxic necrotizing factor 1 and  
lethal toxin on actin cytoskeleton  
and VE-cadherin localization in human endothelial  
cell monolayers.  
AUTHOR: Vouret-Craviari V; Grall D; Flatau G; Pouyssegur J;  
Boquet P; Van Obberghen-Schilling E  
CORPORATE SOURCE: Centre de Biochimie, CNRS UMR 6543, 06108 Nice Cedex  
2, France.  
SOURCE: INFECTION AND IMMUNITY, (1999 Jun) 67 (6) 3002-8.  
Journal code: GO7. ISSN: 0019-9567.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199909  
ENTRY WEEK: 19990901  
AB Integrity of the vascular endothelium is largely dependent on  
endothelial cell shape and establishment of intercellular junctions.  
Certain pathogenic bacterial toxins alter the cytoskeletal  
architecture of intoxicated cells by modulating the GTPase activity  
of p21 Rho family proteins. In the present study we have  
analyzed the effect of Rho-directed toxins on the actin cytoskeleton  
and monolayer integrity of endothelial cells. We report here that  
Escherichia coli cytotoxic necrotizing factor 1 (CNF1) activates Rho  
in human umbilical vein endothelial cells (HUEVC). In confluent  
monolayers, CNF1 treatment induces prominent stress fiber formation  
without significantly modifying peripheral localization of

Searcher : Shears 308-4994

09/126816

VE-cadherin, a specific marker of vascular endothelial cell adherens junctions. Further, Rho activation with CNF1 blocks thrombin-induced redistribution of VE-cadherin staining and gap formation in HUVEC monolayers. Inhibition of Rho by prolonged treatment of cells with C3 exoenzyme (*Clostridium botulinum*) eliminates actin stress fibers without disrupting the continuity of VE-cadherin staining, indicating that Rho-dependent stress fibers are not required for maintaining this adhesion receptor at sites of intercellular contact. **Lethal toxin** (*Clostridium sordellii*), an inhibitor of Rac as well as **Ras** and **Rap**, potently disrupts the actin microfilament system and monolayer integrity in HUVEC cultures.

L7 ANSWER 6 OF 32 MEDLINE DUPLICATE 3  
ACCESSION NUMBER: 1999383591 MEDLINE  
DOCUMENT NUMBER: 99383591  
TITLE: Rundown of somatodendritic N-methyl-D-aspartate (NMDA) receptor channels in rat hippocampal neurones: evidence for a role of the small GTPase RhoA.  
AUTHOR: Norenberg W; Hofmann F; Illes P; Aktories K; Meyer D K  
CORPORATE SOURCE: Department of Pharmacology, Albert-Ludwigs-University, Freiburg, Germany.  
SOURCE: BRITISH JOURNAL OF PHARMACOLOGY, (1999 Jul) 127 (5) 1060-3.  
Journal code: B00. ISSN: 0007-1188.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199912  
ENTRY WEEK: 19991202  
AB Actin filament (F-actin) depolymerization leads to the use-dependent rundown of N-methyl-D-aspartate (NMDA) receptor activity in rat hippocampal neurones. Depolymerization is promoted by Ca<sup>2+</sup> which enters the cells via NMDA receptor channels. The ras homologue (Rho) GTPases (RhoA, Rac1 and Cdc42) promote actin polymerization and thus control the actin cytoskeleton. We have investigated, by means of the whole-cell patch clamp technique, whether the actin fibres which interact with NMDA receptors are controlled by Rho GTPases. In the presence of intracellular ATP which attenuates rundown, the C3 toxin from Clostridium (C.) botulinum was used to inactivate RhoA. Indeed, it enhanced the use-dependent rundown of NMDA-evoked inward currents to a level similar to that obtained in the absence of ATP. Lethal toxin from Clostridium sordellii which inactivates Rac1 and Cdc42 lacked this effect. We suggest that the function of somatodendritic NMDA receptor channels in rat hippocampal neurones can be modulated by RhoA via its action on F-actin.

Searcher : Shears 308-4994

09/126816

L7 ANSWER 7 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)  
ACCESSION NUMBER: 1999:545665 SCISEARCH  
THE GENUINE ARTICLE: 214JE  
TITLE: The actin-based motility of intracellular Listeria  
monocytogenes is not controlled by small GTP-binding  
proteins of the Rho- and Ras-subfamilies  
AUTHOR: Ebel F (Reprint); Rohde M; vonEichelStreiber C;  
Wehland J; Chakraborty T  
CORPORATE SOURCE: UNIV GIESSEN, INST MED MIKROBIOL, FRANKFURTER STR  
107, D-35392 GIESSEN, GERMANY (Reprint); GESELL  
BIOTECHNOL FORSCH MBH, D-3300 BRAUNSCHWEIG, GERMANY;  
UNIV MAINZ, INST MED MIKROBIOL & HYG, D-6500 MAINZ,  
GERMANY  
COUNTRY OF AUTHOR: GERMANY  
SOURCE: FEMS MICROBIOLOGY LETTERS, (1 JUL 1999) Vol. 176,  
No. 1, pp. 117-124.  
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE  
AMSTERDAM, NETHERLANDS.  
ISSN: 0378-1097.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 24

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In this study, we analyzed whether the actin-based motility of  
intracellular Listeria monocytogenes is controlled by the small  
GTP-binding proteins of the Rho- and Ras-subfamilies.  
These signalling proteins are key regulatory elements in the control  
of actin dynamics and their activity is essential for the  
maintenance of most cellular microfilament structures. We used the  
Clostridium difficile toxins TcdB-10463 and TcdB-1470 to  
specifically inactivate these GTP-binding proteins. Treatment of  
eukaryotic cells with either of these toxins led to a dramatic  
breakdown of the normal actin cytoskeleton, but did not abrogate the  
invasion of epithelial cells by L. monocytogenes and had no effect  
on the actin-based motility of this bacterial parasite. Our data  
indicate that intracellular Listeria reorganize the actin  
cytoskeleton in a way that circumvents the control mechanisms  
mediated by the members of the Rho- and Pas-subfamilies that can be  
inactivated by the TcdB-10463 and TcdB-1470 toxins. (C) 1999  
Federation of European Microbiological Societies. Published by  
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L7 ANSWER 8 OF 32 MEDLINE DUPLICATE 4  
ACCESSION NUMBER: 1998344048 MEDLINE  
DOCUMENT NUMBER: 98344048  
TITLE: A common motif of eukaryotic glycosyltransferases is  
essential for the enzyme activity of large  
Searcher : Shears 308-4994

09/126816

AUTHOR: clostridial cytotoxins.  
Busch C; Hofmann F; Selzer J; Munro S; Jeckel D;  
Aktories K

CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie der  
Albert-Ludwigs-Universitat Freiburg,  
Hermann-Herder-Str. 5, D-79104 Freiburg, Germany.

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jul 31) 273  
(31) 19566-72.

PUB. COUNTRY: Journal code: HIV. ISSN: 0021-9258.  
United States  
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199811

ENTRY WEEK: 19981102

AB A fragment of the N-terminal 546 amino acid residues of *Clostridium sordellii* lethal toxin possesses full enzyme activity and glucosylates Rho and Ras GTPases in vitro. Here we identified several amino acid residues in *C. sordellii* lethal toxin that are essential for the enzyme activity of the active toxin fragment. Exchange of aspartic acid at position 286 or 288 with alanine or asparagine decreased glucosyltransferase activity by about 5000-fold and completely blocked glucohydrolase activity. No enzyme activity was detected with the double mutant D286A/D288A. Whereas the wild-type fragment of *C. sordellii* lethal toxin was labeled by azido-UDP-glucose after UV irradiation, mutation of the DXD motif prevented radiolabeling. At high concentrations (10 mM) of manganese ions, the transferase activities of the D286A and D288A mutants but not that of wild-type fragment were increased by about 20-fold. The exchange of Asp270 and Arg273 reduced glucosyltransferase activity by about 200-fold and blocked glucohydrolase activity. The data indicate that the DXD motif, which is highly conserved in all large clostridial cytotoxins and also in a large number of glycosyltransferases, is functionally essential for the enzyme activity of the toxins and may participate in coordination of the divalent cation and/or in the binding of UDP-glucose.

L7 ANSWER 9 OF 32 MEDLINE DUPLICATE 5  
ACCESSION NUMBER: 1998298120 MEDLINE  
DOCUMENT NUMBER: 98298120  
TITLE: Functional consequences of monoglucosylation of Ha-Ras at effector domain amino acid threonine 35.  
AUTHOR: Herrmann C; Ahmadian M R; Hofmann F; Just I  
CORPORATE SOURCE: Max-Planck-Institut fur Molekulare Physiologie,  
Rheinlanddamm 201, D-44139 Dortmund, Germany.  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jun 26) 273  
Searcher : Shears 308-4994

09/126816

(26) 16134-9.

Journal code: HIV. ISSN: 0021-9258.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199810

ENTRY WEEK: 19981002

AB Monoglucosylation of low molecular mass GTPases is an important post-translational modification by which microbes interfere with eukaryotic cell signaling. **Ha-Ras** is monoglucosylated at effector domain amino acid threonine 35 by **Clostridium sordellii** **lethal toxin**, resulting in a blockade of the downstream mitogen-activated protein kinase cascade. To understand the molecular consequences of this modification, effects of glucosylation on each step of the GTPase cycle of **Ras** were analyzed. Whereas nucleotide binding was not significantly altered, intrinsic GTPase activity was markedly decreased, and GTPase stimulation by the GTPase-activating protein p120(GAP) and neurofibromin NF-1 was completely blocked, caused by failure to bind to glucosylated **Ras**. Guanine nucleotide exchange factor (Cdc25)-catalyzed GTP loading was decreased, but not completely inhibited. A dominant-negative property of modified **Ras** to sequester exchange factor was not detectable. However, the crucial step in downstream signaling, **Ras** -effector coupling, was completely blocked. The Kd for the interaction between **Ras.GTP** and the **Ras**-binding domain of **Raf** was 15 nM, whereas glucosylation increased the Kd to >1 mM. Because the affinity of **Ras.GDP** for **Raf** (Kd = 22 &mgr;M) is too low to allow functional interaction, a glucose moiety at threonine 35 of **Ras** seems to block completely the interaction with **Raf**. The net effect of **lethal toxin**-catalyzed glucosylation of **Ras** is the complete blockade of **Ras** downstream signaling.

L7 ANSWER 10 OF 32 MEDLINE

DUPPLICATE 6

ACCESSION NUMBER: 1998184846 MEDLINE

DOCUMENT NUMBER: 98184846

TITLE: Specific inhibition of phorbol ester-stimulated phospholipase D by **Clostridium sordellii** **lethal toxin** and **Clostridium difficile** toxin B-1470 in HEK-293 cells. Restoration by Ral GTPases.

AUTHOR: Schmidt M; Voss M; Thiel M; Bauer B; Grannass A; Tapp E; Cool R H; de Gunzburg J; von Eichel-Streiber C; Jakobs K H

CORPORATE SOURCE: Institut fur Pharmakologie, Universitatsklinikum Essen, D-45122 Essen, Germany.

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Mar 27) 273  
Searcher : Shears 308-4994

(13) 7413-22.

Journal code: HIV. ISSN: 0021-9258.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199806

ENTRY WEEK: 19980604

AB Activation of m<sub>3</sub> muscarinic acetylcholine receptor (mAChR), stably expressed in human embryonic kidney (HEK)-293 cells, leads to phospholipase D (PLD) stimulation, a process apparently involving Rho GTPases, as shown by studies with *Clostridium botulinum* C3 exoenzyme and *Clostridium difficile* toxin B (TcdB). Direct activation of protein kinase C (PKC) by phorbol esters, such as phorbol 12-myristate 13-acetate (PMA), also induces PLD stimulation, which is additive to the mAChR action and which is only poorly sensitive to inactivation of Rho proteins by TcdB. To study whether Ras-like GTPases are involved in PLD regulation, we studied the effects of the TcdB variant TcdB-1470 and *Clostridium sordellii* lethal toxin (TcsL), known to inactivate Rac and some members of the Ras protein family, on PLD activities. TcdB-1470 and TcsL did not affect basal PLD activity and PLD stimulation by mAChR or direct G protein activation. In contrast, PMA-induced PLD stimulation was inhibited by TcdB-1470 and TcsL in a time- and concentration-dependent manner, without alteration in immunologically detectable PKC isozyme levels. In membranes of HEK-293 cells pretreated with TcdB-1470 or TcsL, basal and stable GTP analog-stimulated PLD activities measured with exogenous phosphatidylcholine, in the presence or absence of phosphatidylinositol 4,5-bisphosphate, were not altered. In contrast, pretreatment with TcdB-1470 and TcsL, but not TcdB, strongly reduced PMA-stimulated PLD activity. The addition of recombinant Rac1, serving as glucosylation substrate for TcdB, TcsL, and TcdB-1470, did not restore PLD stimulation by PMA. Furthermore, PMA-stimulated PLD activity, suppressed by prior treatment with TcdB-1470 or TcsL, was not rescued by the addition of recombinant Ras (RasG12V) or Rap proteins, acting as glucosylation substrates for TcsL only (Ras) or TcdB-1470 and TcsL (Rap). In contrast, the addition of recombinant Ral proteins (RalA and RalB), glucosylation substrates for TcsL and TcdB-1470, but not for TcdB, to membranes of TcdB-1470- or TcsL-treated cells fully restored PLD stimulation by PMA without altering the strict MgATP dependence of PMA-induced PLD stimulation. RalA-mediated restoration of PMA-stimulated PLD activity in membranes of TcsL-treated cells was not enhanced by coaddition of RasG12V. In conclusion, the data presented indicate that TcdB-1470 and TcsL selectively interfere with phorbol ester stimulation of PLD and suggest an essential role of Ral proteins in PKC signaling to PLD in HEK-293 cells.

09/126816

L7 ANSWER 11 OF 32 MEDLINE

DUPPLICATE 7

ACCESSION NUMBER: 1998147722 MEDLINE

DOCUMENT NUMBER: 98147722

TITLE: Chimeric clostridial cytotoxins: identification of the N-terminal region involved in protein substrate recognition.

AUTHOR: Hofmann F; Busch C; Aktories K

CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Germany.

SOURCE: INFECTION AND IMMUNITY, (1998 Mar) 66 (3) 1076-81.  
Journal code: G07. ISSN: 0019-9567.

PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199805

ENTRY WEEK: 19980502

AB **Clostridium sordellii** **lethal toxin** is a member of the family of large clostridial cytotoxins that glucosylate small GTPases. In contrast to **Clostridium difficile** toxins A and B, which exclusively modify Rho subfamily proteins, **C. sordellii** **lethal toxin** also glucosylates **Ras** subfamily proteins. By deletion analysis and construction of chimeric fusion proteins of **C. sordellii** **lethal toxin** and **C. difficile** toxin B, we localized the enzyme activity of the **lethal toxin** to the N terminus of the holotoxin and identified the region involved in protein substrate specificity. The toxin fragment of the N-terminal 546 amino acid residues of **C. sordellii** **lethal toxin** glucosylated **Rho** and **Ras** subfamily proteins, as the holotoxin did. Deletion of a further 30 amino acid residues from the C terminus of this active fragment drastically reduced glucotransferase activity and blocked glucohydrolase activity. Exchange of amino acid residues 364 through 516 of **lethal toxin** for those in the active toxin B fragment (1 to 546) allowed glucosylation of **Ras** subfamily proteins. In contrast, the chimera with amino acids 1 to 364 from toxin B, 365 to 468 from **lethal toxin**, and 469 to 546 from toxin B exhibited markedly reduced modification of **Ras** subfamily proteins, whereas modification of **Rac** and **Cdc42** was hardly changed. The data indicate that the region of amino acid residues 364 through 516 primarily defines the substrate specificity of **C. sordellii** **lethal toxin**

L7 ANSWER 12 OF 32 MEDLINE

DUPPLICATE 8

ACCESSION NUMBER: 1998249799 MEDLINE

DOCUMENT NUMBER: 98249799

TITLE: Rho protein inhibition blocks protein kinase C  
Searcher : Shears 308-4994

09/126816

AUTHOR: translocation and activation.  
Hippenstiel S; Kratz T; Krull M; Seybold J; von  
Eichel-Streiber C; Suttorp N  
CORPORATE SOURCE: Department of Internal Medicine, Justus-Liebig-  
University, Giessen, Germany.  
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,  
(1998 Apr 28) 245 (3) 830-4.  
Journal code: 9Y8. ISSN: 0006-291X.

PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199808

ENTRY WEEK: 19980802

AB Small GTP-binding proteins of the **Ras** and Rho family participate in various important signalling pathways. Large clostridial cytotoxins inactivate GTPases by UDP-glucosylation. Using Clostridium difficile toxin B-10463 (TcdB) for inactivation of Rho proteins (RhoA/Rac/Cdc42) and Clostridium **sordellii** **lethal toxin**-1522 (TcsL) for inactivation of **Ras**-proteins (**Ras**/Rac/Ral, Rap) the role of these GTPases in protein kinase C (PKC) stimulation was studied. Phorbol-myristate-acetate (PMA) induced a rapid PKC translocation to and activation in the particulate cell fraction as determined by PKC-activity measurements and Western blots for PKC alpha. These effects were blocked by TcdB inhibiting Rho proteins in endothelial cells, but not in TcsL-treated cells (i.e., cells without **Ras** activity), suggesting that Rho GTPases (RhoA and/or Cdc42) are the most likely GTP-binding proteins responsible for PKC activation. The Rho requirement for PKC activation/translocation was also verified for human epithelial cells and for lipopolysaccharide-stimulated endothelial cells. In summary, the data presented indicate that Rho protein inhibition blocked PKC translocation/activation in endothelial and epithelial cells.

L7 ANSWER 13 OF 32 MEDLINE

DUPLICATE 9

ACCESSION NUMBER: 1999102800 MEDLINE

DOCUMENT NUMBER: 99102800

TITLE: Inhibition of small G proteins by clostridium **sordellii** **lethal toxin**

activates cdc2 and MAP kinase in *Xenopus* oocytes.

AUTHOR: Rime H; Talbi N; Popoff M R; Suziedelis K; Jessus C; Ozon R

CORPORATE SOURCE: INRA/ESA-CNRS 7080, Universite Pierre et Marie Curie,  
4 place Jussieu, 75252 Paris Cedex 05, France.

SOURCE: DEVELOPMENTAL BIOLOGY, (1998 Dec 15) 204 (2) 592-602.  
Journal code: E7T. ISSN: 0012-1606.

PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)

Searcher : Shears 308-4994

09/126816

LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199904  
ENTRY WEEK: 19990401

AB The lethal toxin (LT) from *Clostridium sordellii* is a glucosyltransferase that modifies and inhibits small G proteins of the **Ras** family, **Ras** and **Rap**, as well as **Rac** proteins. LT induces cdc2 kinase activation and germinal vesicle breakdown (GVBD) when microinjected into full-grown *Xenopus* oocytes. Toxin B from *Clostridium difficile*, that glucosylates and inactivates **Rac** proteins, does not induce cdc2 activation, indicating that proteins of the **Ras** family, **Ras** and/or **Rap**, negatively regulate cdc2 kinase activation in *Xenopus* oocyte. In oocyte extracts, LT catalyzes the incorporation of [<sup>14</sup>C]glucose into a group of proteins of 23 kDa and into one protein of 27 kDa. The 23-kDa proteins are recognized by anti-**Rap1** and anti-**Rap2** antibodies, whereas the 27-kDa protein is recognized by several anti-**Ras** antibodies and probably corresponds to **K-Ras**. Microinjection of LT into oocytes together with UDP-[<sup>14</sup>C]glucose results in a glucosylation pattern similar to the in vitro glucosylation, indicating that the 23- and 27-kDa proteins are in vivo substrates of LT. In vivo time-course analysis reveals that the 27-kDa protein glucosylation is completed within 2 h, well before cdc2 kinase activation, whereas the 23-kDa proteins are partially glucosylated at GVBD. This observation suggests that the 27-kDa **Ras** protein could be the in vivo target of LT allowing cdc2 kinase activation. Interestingly, inactivation of **Ras** proteins does not prevent the phosphorylation of c-Raf1 and the activation of MAP kinase that occurs normally around GVBD.

09/126816

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199906

ENTRY WEEK: 19990602

AB Oogonia undergo numerous mitotic cell cycles before completing the last DNA replication and entering the meiotic prophase I. After chromosome pairing and chromatid exchanges between paired chromosomes, the oocyte I remains arrested at the diplotene stage of the first meiotic prophase. Oocyte growth then occurs independently of cell division; indeed, during this growth period, oocytes. (4n DNA) are prevented from completing the meiotic divisions. How is the prophase arrest regulated? One of the players of the prophase block is the high level of intracellular cAMP, maintained by an active adenylate cyclase. By using lethal toxin from *Clostridium sordellii* (LT), a glucosyltransferase that glucosylates and inactivates small G proteins of the Ras subfamily, we have shown that inhibition of either Ras or Rap or both proteins is sufficient to release the prophase block of *Xenopus* oocytes in a cAMP-dependent manner. The implications of Ras family proteins as new players involved in the prophase arrest of *Xenopus* oocytes will be discussed here.

L7 ANSWER 15 OF 32 MEDLINE

DUPLICATE 11

ACCESSION NUMBER: 1999054153 MEDLINE

DOCUMENT NUMBER: 99054153

TITLE: Activation of a Ca<sup>2+</sup>-dependent K<sup>+</sup> current in mouse fibroblasts by lysophosphatidic acid requires a pertussis toxin-sensitive G protein and Ras

AUTHOR: Repp H; Koschinski A; Decker K; Dreyer F

CORPORATE SOURCE: Rudolf-Buchheim-Institut fur Pharmakologie,  
Justus-Liebig-Universitat Giessen, Germany.

SOURCE: NAUNYN-SCHMIEDEBERGS ARCHIVES OF PHARMACOLOGY, (1998  
Nov) 358 (5) 509-17.

PUB. COUNTRY: Journal code: NTQ. ISSN: 0028-1298.

GERMANY: Germany, Federal Republic of  
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199904

ENTRY WEEK: 19990401

AB Lysophosphatidic acid (LPA) is a bioactive lipid that acts through G protein-coupled plasma membrane receptors and mediates a wide range of cellular responses. Here we report that LPA activates a K<sup>+</sup> current in NIH3T3 mouse fibroblasts that leads to membrane hyperpolarization. The activation occurs with an EC<sub>50</sub> value of 1.7 nM LPA. The K<sup>+</sup> current is Ca<sup>2+</sup>-dependent, voltage-independent, and completely blocked by the K<sup>+</sup> channel blockers charybdotoxin, margatoxin, and iberiotoxin with IC<sub>50</sub> values of 1.7, 16, and 62 nM,

Searcher : Shears 308-4994

09/126816

respectively. The underlying K<sub>+</sub> channels possess a single channel conductance of 33 pS in symmetrical K<sub>+</sub> solution. Pretreatment of cells with pertussis toxin (PTX), *Clostridium sordellii* lethal toxin, or a farnesyl protein transferase inhibitor reduced the K<sub>+</sub> current amplitude in response to LPA to about 25% of the control value. Incubation of cells with the protein tyrosine kinase inhibitor genistein or microinjection of the neutralizing anti-Ras monoclonal antibody Y13-259 reduced it by more than 50%. In contrast, the phospholipase C inhibitor U-73122 and the protein kinase A activator 8-bromo-cAMP had no effect. These results indicate that the K<sub>+</sub> channel activation by LPA is mediated by a signal transduction pathway involving a PTX-sensitive G protein, a protein tyrosine kinase, and Ras. LPA is already known to activate Cl<sub>-</sub> channels in various cell types, thereby leading to membrane depolarization. In conjunction with our results that demonstrate LPA-induced membrane hyperpolarization by activation of K<sub>+</sub> channels, LPA appears to be significantly involved in the regulation of the cellular membrane potential.

L7 ANSWER 16 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)  
ACCESSION NUMBER: 1999:475976 SCISEARCH  
THE GENUINE ARTICLE: BN14K  
TITLE: Activation and inactivation of Ras-like GTPases by bacterial cytotoxins  
AUTHOR: vonEichelStreiber C (Reprint); Weidmann M; Giry M; Moos M  
CORPORATE SOURCE: INST MED MIKROBIOL & HYG, VERFUGUNGSGEBDUE FORSCH & ENTWICKLUNG, OBERE ZAHLBACHERSTR 63, D-55101 MAINZ, GERMANY (Reprint)  
COUNTRY OF AUTHOR: GERMANY  
SOURCE: METHODS IN MICROBIOLOGY, (JUN 1998) Vol. 27, pp. 509-525.  
Publisher: ACADEMIC PRESS LTD, 24-28 OVAL ROAD, LONDON NW1 7DX, ENGLAND.  
ISSN: 0580-9517.  
DOCUMENT TYPE: General Review; Journal  
FILE SEGMENT: LIFE; AGRI  
LANGUAGE: English  
REFERENCE COUNT: 54

L7 ANSWER 17 OF 32 MEDLINE DUPLICATE 12  
ACCESSION NUMBER: 1998267059 MEDLINE  
DOCUMENT NUMBER: 98267059  
TITLE: Activation of a Ca<sup>2+</sup>-dependent K<sub>+</sub> current by the oncogenic receptor protein tyrosine kinase v-Fms in mouse fibroblasts.  
AUTHOR: Decker K; Koschinski A; Trouliaris S; Tamura T; Dreyer F; Repp H  
Searcher : Shears 308-4994

09/126816

CORPORATE SOURCE: Rudolf-Buchheim-Institut fur Pharmakologie,  
Justus-Liebig-Universitat Giessen, Germany.  
SOURCE: NAUNYN-SCHMIEDEBERGS ARCHIVES OF PHARMACOLOGY, (1998  
Apr) 357 (4) 378-84.  
Journal code: NTQ. ISSN: 0028-1298.  
PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199810  
ENTRY WEEK: 19981001

AB We investigated the effects of the receptor-coupled protein tyrosine kinase (RTK) v-Fms on the membrane current properties of NIH3T3 mouse fibroblasts. We found that v-Fms, the oncogenic variant of the macrophage colony-stimulating factor receptor c-Fms, activates a K<sup>+</sup> current that is absent in control cells. The activation of the K<sup>+</sup> current was Ca<sup>2+</sup>-dependent, voltage-independent, and was completely blocked by the K<sup>+</sup> channel blockers charybdotoxin, margatoxin and iberiotoxin with IC<sub>50</sub> values of 3 nM, 18 nM and 76 nM, respectively. To identify signalling components that mediate the activation of this K<sup>+</sup> current, NIH3T3 cells that express different mutants of the wild-type v-Fms receptor were examined. Mutation of the binding site for the Ras-GTPase-activating protein led to a complete abolishment of the K<sup>+</sup> current. A reduction of 76% and 63%, respectively, was observed upon mutation of either of the two binding sites for the growth factor receptor binding protein 2. Mutation of the ATP binding lobe, which disrupts the protein tyrosine kinase activity of v-Fms, led to a 55% reduction of the K<sup>+</sup> current. Treatment of wild-type v-Fms cells with Clostridium sordellii lethal toxin or a farnesyl protein transferase inhibitor, both known to inhibit the biological function of Ras, reduced the K<sup>+</sup> current amplitude to 17% and 6% of the control value, respectively. This is the first report showing that an oncogenic RTK can modulate K<sup>+</sup> channel activity. Our results indicate that this effect is dependent on the binding of certain Ras-regulating proteins to the v-Fms receptor and is not abolished by disruption of its intrinsic protein tyrosine kinase activity. Furthermore, our data suggest that Ras plays a key role for K<sup>+</sup> channel activation by the oncogenic RTK v-Fms.

L7 ANSWER 18 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)  
ACCESSION NUMBER: 1998:456658 SCISEARCH  
THE GENUINE ARTICLE: ZT483  
TITLE: Genetic rearrangements in the pathogenicity locus of Clostridium difficile strain 8864 - implications for transcription, expression and enzymatic activity of toxins A and B  
AUTHOR: Soehn F; WagenknechtWiesner A; Leukel P; Kohl M;  
Searcher : Shears 308-4994

09/126816

CORPORATE SOURCE: Weidmann M; vonEichelStreiber C (Reprint); Braun V  
JOHANNES GUTENBERG UNIV, INST MED MIKROBIOL & HYG,  
VERFUGUNGSGEBAUDE FORSCH & ENTWICKLUNG, D-55101  
MAINZ, GERMANY (Reprint); JOHANNES GUTENBERG UNIV,  
INST MED MIKROBIOL & HYG, VERFUGUNGSGEBAUDE FORSCH &  
ENTWICKLUNG, D-55101 MAINZ, GERMANY  
GERMANY  
COUNTRY OF AUTHOR:  
SOURCE: MOLECULAR & GENERAL GENETICS, (MAY 1998) Vol. 258,  
No. 3, pp. 222-232.  
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK,  
NY 10010.  
ISSN: 0026-8925.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 36

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The pathogenicity locus (PaLoc) of *Clostridium difficile* isolate 8864 was investigated to locate genetic rearrangements that would explain the exceptional pathogenicity of this particular isolate. Two major changes were defined: an insertion of 1.1 kb between the two genes *tcdA* and *tcdE*, coding for the enterotoxin and an accessory protein of unknown function, respectively, and a deletion of 5.9 kb encompassing the 3' ends of *tcdA* and *tcdC*. Transcription of the *tcdA-E* genes is severely affected by both rearrangements, explaining the demonstrated complete lack of TcdA polypeptide. We present a model of coordinate, growth-related transcription of the *tcdA-E* genes that confirms our previous findings in strain 10463. Recombinant TcdA-8864 had UDP-glucose-glucosyltransferase activity, proving that the N-terminal 698 amino acids of the polypeptide represent the catalytic domain. However, this truncated TcdA molecule lacks a ligand and translocation domain. To assess the catalytic domain of TcdB-8864, the sequence of the 5' end of its gene was determined. TcdB-8864 shows high homology to TcdB-1470 but lower homology to TcdB-10463 within this domain. This fits well with the altered glucosylation specificity of TcdB-8864 (Rac1, Rap2 and Ral). Having defined the variations of transcription, expression and enzymatic activity of toxins A and B, implications for the pathogenic potential of strain 8864 are discussed.

L7 ANSWER 19 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)  
ACCESSION NUMBER: 1998:891246 SCISEARCH  
THE GENUINE ARTICLE: 136CD  
TITLE: Inhibition of p38 and p42/p44 MAPK by *Clostridium sordellii* lethal toxin  
in IL-1 stimulated T lymphocytes - A role for a  
Ras subfamily G protein in IL-1 signalling  
AUTHOR: Palsson E M (Reprint); Popoff M R; Thelestam M;  
ONeill L A J.  
Searcher : Shears 308-4994

09/126816

CORPORATE SOURCE: TRINITY COLL DUBLIN, DEPT BIOCHEM, DUBLIN, IRELAND;  
INST PASTEUR, F-75724 PARIS, FRANCE; KAROLINSKA  
INST, S-17111 STOCKHOLM, SWEDEN

COUNTRY OF AUTHOR: IRELAND; FRANCE; SWEDEN

SOURCE: EUROPEAN CYTOKINE NETWORK, (SEP 1998) Vol. 9, No. 3,  
pp. 129-129.

Publisher: JOHN LIBBEY EUROTEXT LTD, 127 AVE DE LA  
REPUBLIQUE, 92120 MONTROUGE, FRANCE.

ISSN: 1148-5493.

DOCUMENT TYPE: Conference; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 0

L7 ANSWER 20 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)

ACCESSION NUMBER: 1998:427521 SCISEARCH

THE GENUINE ARTICLE: ZQ716

TITLE: Signalling through small GTPases

AUTHOR: Mattingly R R (Reprint)

CORPORATE SOURCE: UNIV VIRGINIA, MARKEY CTR CELL SIGNALLING, BOX 577,  
CHARLOTTESVILLE, VA 22908 (Reprint)

COUNTRY OF AUTHOR: USA

SOURCE: IN VITRO & MOLECULAR TOXICOLOGY-A JOURNAL OF BASIC  
AND APPLIED RESEARCH, (SPR 1998) Vol. 11, No. 1, pp.  
57-62.

Publisher: MARY ANN LIEBERT INC PUBL, 2 MADISON  
AVENUE, LARCHMONT, NY 10538.

ISSN: 1097-9336.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 33

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The **Ras** superfamily of small GTPases provides a ubiquitous control mechanism for many cellular processes. Regions outside of the highly-conserved GTPase core domain are variable and show induced conformational changes as the protein cycles between GTP-and GDP-bound states. These sequence variations define several subfamilies of small GTPases that show similar functions. For example, **Ras** proteins control cell growth, Rab proteins direct vesicle fusion, Ran is essential for nuclear protein transport, and Rac/Rho proteins organize the actin cytoskeleton.

Damage to these small GTPases can have catastrophic consequences for the cell and organism. Several Rac/Rho subfamily members are direct targets for clostridial cytotoxins. Further, **Ras** proteins are mutated to a constitutively-active form in approximately 20% of human cancers.

Physiological control of these GTPases switches occurs through exchange factors that catalyze the conversion to the GTP-bound "on" state and through GAPs (GTPase-activating proteins) that

Searcher : Shears 308-4994

09/126816

accelerate the GTPase activity and the return to the GDP-bound 'off' state. Recent work has identified anew pathway for the activation of Ras through phosphorylation of an exchange factor called Ras-GRF. Stimulation of receptors coupled to heterotrimeric G-proteins can lead, via a G beta gamma-dependent pathway, to an increase in the specific activity of Ras-GRF toward Ras.

L7 ANSWER 21 OF 32 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD  
ACCESSION NUMBER: 1997-402313 [37] WPIDS  
DOC. NO. CPI: C1997-129748  
TITLE: Use of *Clostridium sordellii*  
lethal toxin - for inactivating  
Ras by glucosylation, used for treating  
conditions such as cancer, particularly pancreatic  
or colon cancer.  
DERWENT CLASS: B04 D16  
INVENTOR(S): BOQUET, P; THELESTAM, M; VON EICHELSTREIBER, C; VON  
EICHEL-STREIBER, C  
PATENT ASSIGNEE(S): (BOEFL) BOEHRINGER MANNHEIM GMBH; (ASTA) ASTA MEDICA  
AG  
COUNTRY COUNT: 75  
PATENT INFORMATION:

| PATENT NO   | KIND | DATE               | WEEK | LA | PG |
|---|------|--------------------|------|----|----|
| WO 9727871  | A1   | 19970807 (199737)* | EN   | 45 |    |
| RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA<br>PT SD SE SZ UG   |      |                    |      |    |    |
| W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI<br>GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG<br>MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA<br>UG US UZ VN |      |                    |      |    |    |
| AU 9715982  | A    | 19970822 (199801)  |      |    |    |
| EP 877622   | A1   | 19981118 (199850)  | EN   |    |    |
| R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE  |      |                    |      |    |    |

APPLICATION DETAILS:

| PATENT NO  | KIND | APPLICATION    | DATE     |
|------------|------|----------------|----------|
| WO 9727871 | A1   | WO 1997-EP426  | 19970131 |
| AU 9715982 | A    | AU 1997-15982  | 19970131 |
|            |      | WO 1997-EP426  | 19970131 |
| EP 877622  | A1   | EP 1997-902278 | 19970131 |
|            |      | WO 1997-EP426  | 19970131 |

FILING DETAILS:

Searcher : Shears 308-4994

09/126816

| PATENT NO  | KIND        | PATENT NO  |
|------------|-------------|------------|
| AU 9715982 | A Based on  | WO 9727871 |
| EP 877622  | A1 Based on | WO 9727871 |

PRIORITY APPLN. INFO: EP 1996-101469 19960202

AN 1997-402313 [37] WPIDS

AB WO 9727871 A UPAB: 19970915

An immunotoxin (A) comprises a first, second and third part, connected by covalent bonds and a pharmaceutically acceptable carrier: (a) the first part includes a target cell specific binding domain, which is able to cause the **LT** (lethal toxin) immunotoxin of **Clostridium sordellii** (CS) to bind to the patient's cell; (b) the second part includes a translocation domain of a protein capable of translocating the third part across the cytoplasmic membrane of the cell; and (c) the third part includes a polypeptide with the toxic activity of the catalytic domain of **LT** from CS.

A composition for the treatment of a pathological disorder associated with the activation of **Ras proto**-oncoproteins comprising (A) and a pharmaceutically acceptable carrier is also claimed.

USE - The CS LT can inactivate **Ras** by glucosylation of **Ras** threonine 35. The products can be used for treating cancers, particularly pancreas or colon cancer.

Dwg.0/7

L7 ANSWER 22 OF 32 TOXLIT

ACCESSION NUMBER: 1997:129518 TOXLIT

DOCUMENT NUMBER: CA-127-195467D

TITLE: Immunotoxin inactivation of **Ras** subfamily proteins and agents therefor.

AUTHOR: Von Eichel-Streiber C; Boquet P; Thelestam M

SOURCE: (1997). PCT Int. Appl. PATENT NO. 9727871 08/07/1997 (Thelestam, Monica).

CODEN: PIXXD2.

PUB. COUNTRY: GERMANY, FEDERAL REPUBLIC OF

DOCUMENT TYPE: Patent

FILE SEGMENT: CA

LANGUAGE: English

OTHER SOURCE: CA 127:195467

ENTRY MONTH: 199805

AB The invention comprises a method of treating a patient with a disorder, characterized by an activating mutation in the **Ras** proto-oncogene, comprising contacting cells of said patient with a protein having the toxic activity of **Clostridium sordellii** toxin **LT** under conditions favoring inactivating of **Ras** by glucosylation of **Ras** threonine 35 in said cell. Said protein preferably is

Searcher : Shears 308-4994

09/126816

an immunotoxin which contains as a toxic domain the catalytic domain of toxin LT.

L7 ANSWER 24 OF 32 MEDLINE DUPLICATE 14  
Searcher : Shears 308-4994

09/126816

ACCESSION NUMBER: 1998124099 MEDLINE  
DOCUMENT NUMBER: 98124099  
TITLE: Evidence for differential roles of the Rho subfamily  
of GTP-binding proteins in glucose- and  
calcium-induced insulin secretion from pancreatic  
beta cells.  
AUTHOR: Kowluru A; Li G; Rabaglia M E; Segu V B; Hofmann F;  
Aktories K; Metz S A  
CORPORATE SOURCE: Medical and Research Services, William S. Middleton  
Memorial VA Medical Center, Madison, WI 53705, USA..  
akowluru@facstaff.wisc.edu  
CONTRACT NUMBER: DK 37312 (NIDDK)  
SOURCE: BIOCHEMICAL PHARMACOLOGY, (1997 Nov 15) 54 (10)  
1097-108.  
Journal code: 9Z4. ISSN: 0006-2952.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199804  
ENTRY WEEK: 19980403

AB We utilized clostridial **toxins** (with known specificities for inhibition of GTPases) to ascertain the contribution of candidate GTPases in physiologic insulin secretion from beta cells. Exposure of normal rat islets or isolated beta (HIT-T15) cells to *Clostridium difficile* **toxins** A and B catalyzed the glucosylation (and thereby the inactivation) of Rac, Cdc42, and Rho endogenous to beta cells; concomitantly, either **toxin** reduced glucose- or potassium-induced insulin secretion from rat islets and HIT cells. Treatment of beta cells with *Clostridium sordellii* lethal **toxin** (LT, which modified only Ras, Rap, and Rac) also reduced glucose- or potassium-induced secretion. However, clostridial **toxin** C3-exoenzyme (which ADP-ribosylates and inactivates only Rho) was without any effect on either glucose- or potassium-induced insulin secretion. These data suggest that Cdc42, Rac, Ras, and/or Rap (but not Rho) may be needed for glucose- or potassium-mediated secretion. The effects of these **toxins** appear to be specific on stimulus-secretion coupling, since no difference in metabolic viability (assessed colorimetrically by quantitating the conversion of the tetrazolium salt into a formazan in a reduction reaction driven by nutrient metabolism) was demonstrable between control and **toxin** (A or LT)-treated beta cells. **Toxin** (A or LT) treatment also did not alter glucose- or potassium-mediated rises in cytosolic free calcium concentrations ( $[Ca^{2+}]_i$ ), suggesting that these GTPases are involved in steps distal to elevations in  $[Ca^{2+}]_i$ . Recent findings indicate that the carboxyl methylation of Cdc42 is stimulated by only glucose, whereas

Searcher : Shears 308-4994

09/126816

that of Rap (Kowluru et al., J Clin Invest 98: 540-555, 1996) and Rac (present study) are regulated by glucose or potassium. Together, these findings provide direct evidence, for the first time, that the Rho subfamily of GTPases plays a key regulatory role(s) in insulin secretion, and they suggest that Cdc42 may be required for early steps in glucose stimulation of insulin release, whereas Rap and/or Rac may be required for a later step(s) in the stimulus-secretion coupling cascade (i.e.  $Ca^{2+}$ -induced exocytosis of insulin).

Searcher : Shears 308-4994

09/126816

L7 ANSWER 26 OF 32 MEDLINE DUPLICATE 16  
ACCESSION NUMBER: 96215306 MEDLINE  
DOCUMENT NUMBER: 96215306  
TITLE: Inactivation of Ras by Clostridium  
sordellii lethal toxin  
-catalyzed glucosylation.  
AUTHOR: Just I; Selzer J; Hofmann F; Green G A; Aktories K  
CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie der  
Universitat Freiburg, Hermann-Herder-Strasse 5,  
D-79104 Freiburg, Germany.  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Apr 26) 271  
(17) 10149-53.  
Journal code: HIV. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199608  
AB The lethal toxin (LT) from Clostridium  
sordellii belongs to the family of large clostridial  
cytotoxins causing morphological alterations in cultured cell lines  
accompanied by destruction of the actin cytoskeleton. C.  
sordellii LT exhibits 90% homology to Clostridium  
difficile toxin B, which has been recently identified as a  
monoglucosyltransferase (Just, I., Selzer, J., Wilm, M., von  
Eichel-Streiber, C., Mann, M., and Aktories, K. (1995) Nature 375,  
500-503). We report here that LT too is a  
glucosyltransferase, which uses UDP-glucose as cosubstrate to modify  
low molecular mass GTPases. LT selectively modifies Rac  
and Ras, whereas the substrate specificity of  
toxin B is confined to the Rho subfamily proteins Rho, Rac,  
and Cdc42, which participate in the regulation of the actin  
cytoskeleton. In Rac, both toxin B and LT share  
the same acceptor amino acid, threonine 35. Glucosylation of  
Ras by LT results in inhibition of the epidermal  
growth factor-stimulated p42/p44 MAP-kinase signal pathway.  
LT is the first bacterial toxin to inactivate  
Ras in intact cells.

L7 ANSWER 27 OF 32 BIOSIS COPYRIGHT 1999 BIOSIS  
ACCESSION NUMBER: 1996:292456 BIOSIS  
DOCUMENT NUMBER: PREV199699014812  
TITLE: Ca-2+ channel activation by platelet-derived growth factor-induced tyrosine phosphorylation and Ras guanine triphosphate-binding proteins in rat glomerular mesangial cells.  
AUTHOR(S): Ma, Heping; Matsunaga, Hiroshi; Li, Bing; Schieffer, Bernhard; Marrero, Mario B.; Ling, Brian N. (1)  
CORPORATE SOURCE: (1) Emory Univ. Sch. Med., Renal Div., 1364 Clifton  
Searcher : Shears 308-4994

09/126816

Road N.E., Atlanta, GA 30322 USA  
SOURCE: Journal of Clinical Investigation, (1996) Vol. 97,  
No. 10, pp. 2332-2341.  
ISSN: 0021-9738.

DOCUMENT TYPE: Article  
LANGUAGE: English

AB We investigated the signaling pathways mediating 1-pS Ca-2+ channel activation by PDGF in cultured rat mesangial cells. In cell-attached patches, intrapipette PDGF-BB (PDGF B chain homodimer isoform) (50 ng/ml) dramatically stimulates channel activity (P  $\leq$  0.003, n = 6). Tyrosine kinase inhibition (100  $\mu$ M genistein or 10  $\mu$ M tryphostin 9) abolished PDGF-induced channel activation (P  $\leq$  0.02, n = 6). In excised patches, the effect of tyrosine kinase inhibition could be reversed by 200  $\mu$ M GTP-gamma-S (P  $\leq$  0.02, n = 4). In contrast, 200  $\mu$ M GDP-beta-S inhibited PDGF-induced channel activity (P  $\leq$  0.04, n = 6). Pertussis toxin (250 ng/ml) had no effect on PDGF-induced channel activity (P = 0.45, n = 6). When excised patches were exposed to anti-Ras antibody (5  $\mu$ g/ml), PDGF-induced channel activity was abolished (P  $\leq$  0.002, n = 11). Western immunoblots revealed that PDGF-BB binding stimulates the formation of a membrane-bound complex consisting of growth factor receptor-binding protein 2, son of sevenless, and the PDGF-beta receptor. Complex formation was abolished by genistein. In mesangial cells, the intrinsic tyrosine kinase activity of the PDGF-beta receptor stimulates the formation of a membrane-bound growth factor receptor-binding protein 2/son of sevenless/PDGF-beta receptor complex and activation of the pertussis toxin-insensitive GTP-binding protein, p21-Ras, which leads to the opening of 1-pS Ca-2+ channels.

L7 ANSWER 28 OF 32 SCISEARCH COPYRIGHT 1999 ISI (R)

ACCESSION NUMBER: 96:754123 SCISEARCH

THE GENUINE ARTICLE: VL996

TITLE: LARGE CLOSTRIDIAL CYTOTOXINS - A FAMILY OF GLYCOSYLTRANSFERASES MODIFYING SMALL GTP-BINDING PROTEINS

AUTHOR: VONEICHELSTREIBER C (Reprint); BOQUET P; SAUERBORN M; THELESTAM M

CORPORATE SOURCE: UNIV MAINZ, INST MED MIKROBIOL & HYG, D-55101 MAINZ, GERMANY (Reprint); FAC MED NICE, INSERM, U452, F-06107 NICE 2, FRANCE; CCLRC DARESBURY LAB, SYNCHROTRON RADIAT DEPT, WARRINGTON WA4 4AD, CHESHIRE, ENGLAND; KAROLINSKA INST, CTR MICROBIOL & TUMORBIOL, S-17177 STOCKHOLM, SWEDEN

COUNTRY OF AUTHOR: GERMANY; FRANCE; ENGLAND; SWEDEN

SOURCE: TRENDS IN MICROBIOLOGY, (OCT 1996) Vol. 4, No. 10, pp. 375-382.

ISSN: 0966-842X.

Searcher : Shears 308-4994

09/126816

DOCUMENT TYPE: Article; Journal  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 55

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Some Clostridium species produce AB(x)-type protein cytotoxins of high molecular weight. These toxins constitute the group of large clostridial cytotoxins (LCTs), which have homologous protein sequences, exert glycosyltransferase activity and modify GTP-binding proteins of the Ras-superfamily. These characteristics render the LCTs valuable tools for developmental and cell biologists.

L7 ANSWER 29 OF 32 MEDLINE DUPLICATE 17  
ACCESSION NUMBER: 97127410 MEDLINE  
DOCUMENT NUMBER: 97127410  
TITLE: Difference in protein substrate specificity between hemorrhagic toxin and lethal toxin from Clostridium sordellii.  
AUTHOR: Genth H; Hofmann F; Selzer J; Rex G; Aktories K; Just I  
CORPORATE SOURCE: Institut fur Pharmakologie und Toxikologie der Albert-Ludwigs-Universitat Freiburg, Germany.  
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1996 Dec 13) 229 (2) 370-4.  
Journal code: 9Y8. ISSN: 0006-291X.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199703  
ENTRY WEEK: 19970304

AB The hemorrhagic toxin (HT) from Clostridium sordellii is pharmacologically related to Clostridium difficile toxins A and B and Clostridium sordellii lethal toxin which have been recently identified as mono-glucosyl-transferases. Here we report that HT, which is coexpressed with lethal toxin, is also a glucosyltransferase. Whereas lethal toxin glucosylates the Rho subfamily proteins Rac and Cdc42 and the Ras subfamily proteins H-Ras and Rap, the substrate specificity of HT is strictly confined to the Rho subfamily proteins Rho, Rac and Cdc42. Comparable to lethal toxin, transferase activity of HT is stimulated by Mn<sup>2+</sup>. Acceptor amino acid in Rho was identified by mutagenesis as threonine-37. C. sordellii HT is a novel member of the family of clostridial mono-glucosyl-transferases, a family which modifies the Rho and Ras GTPases.

L7 ANSWER 30 OF 32 MEDLINE DUPLICATE 18  
ACCESSION NUMBER: 97011096 MEDLINE  
Searcher : Shears 308-4994

09/126816

DOCUMENT NUMBER: 97011096  
TITLE: The ras-related protein Ral is  
monoglycosylated by Clostridium sordellii  
lethal toxin.  
AUTHOR: Hofmann F; Rex G; Aktories K; Just I  
CORPORATE SOURCE: Institut fur Pharmakologie and Toxikologie,  
Albert-Ludwigs-Universitat Freiburg, Germany.  
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,  
(1996 Oct 3) 227 (1) 77-81.  
Journal code: 9Y8. ISSN: 0006-291X.  
PUB. COUNTRY: United States  
Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199701  
ENTRY WEEK: 19970104  
AB Clostridium sordellii lethal toxin (LT), a cytotoxin which causes preferential destruction of the actin cytoskeleton, has been recently identified as glucosyltransferase to modify the low molecular mass GTPases Rac, Ras and Rap. We report here on LT produced by C. sordellii strain 6018 which glucosylates in addition to Rac, Ras and Rap the Ral protein. LT from strain VPI19048 however does not glucosylate Ral. Besides recombinant Ral, cellular Ral is also substrate. In the GDP-bound form, Ral is a superior substrate to the GTP form. Acceptor amino acid for glucose is threonine-46 which is equivalent to threonine-35 in H-Ras located in the effector region. The Ral-glucosylating toxin is a novel isoform of Ras-modifying clostridial cytotoxins.

L7 ANSWER 31 OF 32 BIOSIS COPYRIGHT 1999 BIOSIS  
ACCESSION NUMBER: 1996:304294 BIOSIS  
DOCUMENT NUMBER: PREV199699026650  
TITLE: Inactivation of Ras by glucosylation  
catalyzed by Clostridium sordellii  
lethal toxin.  
AUTHOR(S): Just, I. (1); Selzer, J. (1); Kern, O. (1); Green, G.  
A.; Aktories, K. (1)  
CORPORATE SOURCE: (1) Inst. Pharmakologie Toxikologie, Univ. Freiburg,  
D-79104 Freiburg Germany  
SOURCE: Naunyn-Schmiedeberg's Archives of Pharmacology,  
(1996) Vol. 353, No. 4 SUPPL., pp. R19.  
Meeting Info.: 37th Spring Meeting of the German  
Society for Experimental and Clinical Pharmacology  
and Toxicology Mainz, Germany March 12-14, 1996  
ISSN: 0028-1298.  
DOCUMENT TYPE: Conference  
LANGUAGE: English

Searcher : Shears 308-4994

09/126816

L7 ANSWER 32 OF 32 TOXLINE  
ACCESSION NUMBER: 1996:71418 TOXLINE  
DOCUMENT NUMBER: BIOSIS-96-22459  
TITLE: INACTIVATION OF RAS BY GLUCOSYLATION  
CATALYZED BY CLOSTRIDIUM SORDELLII  
LETHAL TOXIN.  
AUTHOR: JUST I; SELZER J; KERN O; GREEN G A; AKTORIES K  
SOURCE: (1996). Vol. 353, No. 4 SUPPL:R19. 37TH SPRING  
MEETING OF THE GERMAN SOCIETY FOR EXPERIMENTAL AND  
CLINICAL PHARMACOLOGY AND TOXICOLOGY, MAINZ, GERMANY,  
MARCH 12-14, 1996. NAUNYN-SCHMIEDEBERG'S ARCHIVES OF  
PHARMACOLOGY.  
CODEN: NSAPCC.  
FILE SEGMENT: BIOSIS  
LANGUAGE: English  
ENTRY MONTH: 199608  
AB BIOSIS COPYRIGHT: BIOL ABS. RRM MEETING ABSTRACT  
CLOSTRIDIUM-DIFFICILE HUMAN THREONINE

FILE 'USPATFULL' ENTERED AT 11:45:40 ON 16 NOV 1999  
L8 2 S L5

L8 ANSWER 1 OF 2 USPATFULL  
ACCESSION NUMBER: 1999:24634 USPATFULL  
TITLE: Enhancer sequence for modulating expression in  
epithelial cells  
INVENTOR(S): Kufe, Donald, Wellesley, MA, United States  
Abe, Miyako, Boston, MA, United States  
PATENT ASSIGNEE(S): Dana-Farber Cancer Institute, Inc., Boston, MA,  
United States (U.S. corporation)

|  | NUMBER  | DATE         |
|--|---|--------------|
| PATENT INFORMATION:                        | US 5874415  | 19990223     |
| APPLICATION INFO.:                         | US 1995-465981  | 19950606 (8) |
| RELATED APPLN. INFO.:                      | Division of Ser. No. US 1994-324465, filed on 17<br>Oct 1994, now patented, Pat. No. US 5565334 which<br>is a continuation of Ser. No. US 1992-999742,<br>filed on 31 Dec 1992, now abandoned |              |
| DOCUMENT TYPE:                             | Utility   |              |
| PRIMARY EXAMINER:                          | Degen, Nancy  |              |
| LEGAL REPRESENTATIVE:                      | Fish & Richardson P.C.  |              |
| NUMBER OF CLAIMS:                          | 20  |              |
| EXEMPLARY CLAIM:                           | 1   |              |
| NUMBER OF DRAWINGS:                        | 10 Drawing Figure(s); 8 Drawing Page(s)   |              |
| LINE COUNT:                                | 863   |              |
| CAS INDEXING IS AVAILABLE FOR THIS PATENT. |   |              |
| AB   | Isolated DNA encompassing the DF3 enhancer as well as a sequence<br>Searcher : Shears 308-4994  |              |

09/126816

encoding a heterologous polypeptide provides epithelial tissue-selective gene expression of the heterologous polypeptide, useful in methods of therapy.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 514/044.000  
INCLS: 514/002.000; 536/024.100; 536/024.500  
NCL NCLM: 514/044.000  
NCLS: 514/002.000; 536/024.100; 536/024.500

L8 ANSWER 2 OF 2 USPATFULL

ACCESSION NUMBER: 96:94465 USPATFULL  
TITLE: Enhancer sequence for modulating expression in epithelial cells  
INVENTOR(S): Kufe, Donald, Wellesley, MA, United States  
Abe, Miyako, Boston, MA, United States  
PATENT ASSIGNEE(S): Dana-Farber Cancer Institute, Inc., Boston, MA, United States (U.S. corporation)

|                       | NUMBER   | DATE         |
|-----------------------|--|--------------|
| PATENT INFORMATION:   | US 5565334   | 19961015     |
| APPLICATION INFO.:    | US 1994-324465   | 19941017 (8) |
| RELATED APPLN. INFO.: | Continuation of Ser. No. US 1992-999742, filed on 31 Dec 1992, now abandoned |              |
| DOCUMENT TYPE:        | Utility  |              |
| PRIMARY EXAMINER:     | Elliott, George C.   |              |
| LEGAL REPRESENTATIVE: | Fish & Richardson P.C.   |              |
| NUMBER OF CLAIMS:     | 25   |              |
| EXEMPLARY CLAIM:      | 1  |              |
| NUMBER OF DRAWINGS:   | 10 Drawing Figure(s); 8 Drawing Page(s)                                      |              |
| LINE COUNT:           | 867  |              |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Isolated DNA encompassing the DF3 enhancer as well as a sequence encoding a heterologous polypeptide provides epithelial tissue-selective gene expression of the heterologous polypeptide, useful in methods of therapy.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/069.100  
INCLS: 435/240.200; 435/320.100; 536/023.100; 536/023.200;  
536/024.100; 536/024.500  
NCL NCLM: 435/069.100  
NCLS: 435/320.100; 435/371.000; 536/023.100; 536/023.200;  
536/024.100; 536/024.500

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, PROMT, TOXLIT, TOXLINE, CANCERLIT, USPATFULL' ENTERED AT 11:46:17 ON 16 NOV 1999)

Searcher : Shears 308-4994

09/126816

-Author(s)

L9 20 SEA ABB=ON PLU=ON (EICHEL STREIBER C? OR STREIBER  
EICHEL C? OR STREIBER C? OR EICHEL C?)/AU  
L10 1022 SEA ABB=ON PLU=ON BOQUET P?/AU  
L11 728 SEA ABB=ON PLU=ON THELESTAM M?/AU  
L12 0 SEA ABB=ON PLU=ON L9 AND L10 AND L11  
L13 3 SEA ABB=ON PLU=ON L9 AND (L10 OR L11)  
L14 27 SEA ABB=ON PLU=ON L10 AND L11  
L15 79 SEA ABB=ON PLU=ON (L9 OR L10 OR L11) AND RAS  
L16 93 SEA ABB=ON PLU=ON L13 OR L14 OR L15  
L17 24 DUP REM L16 (69 DUPLICATES REMOVED)

L17 ANSWER 1 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1  
ACCESSION NUMBER: 1999:351907 CAPLUS  
DOCUMENT NUMBER: 131:98722  
TITLE: G-protein-stimulated phospholipase D activity is  
inhibited by lethal toxin from Clostridium  
sordellii in HL-60 cells  
AUTHOR(S): El Hadj, Noomen Ben; Popoff, Michel R.; Marvaud,  
Jean-Christophe; Payrastre, Bernard;  
Boquet, Patrice; Geny, Blandine  
CORPORATE SOURCE: INSERM U332, ICGM, Paris, 75014, Fr.  
SOURCE: J. Biol. Chem. (1999), 274(20), 14021-14031  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular  
Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Lethal toxin (LT) from Clostridium sordellii has been shown in HeLa  
cells to glucosylate and inactivate Ras and Rac and,  
hence, to disorganize the actin cytoskeleton. In the present work,  
we demonstrate that LT treatment provokes the same effects in HL-60  
cells. We show that guanosine 5'-O-(3-thiotriphosphate)-stimulated  
phospholipase D (PLD) activity is inhibited in a time- and  
dose-dependent manner after an overnight treatment with LT. A  
similar dose response to the toxin was found when PLD activity was  
stimulated by phorbol 12-myristate 3-acetate via the protein kinase  
C pathway. The toxin effect on actin organization seemed unlikely  
to account directly for PLD inhibition as cytochalasin D and iota  
toxin from Clostridium perfringens E disorganize the actin  
cytoskeleton without modifying PLD activity. However, the enzyme  
inhibition and actin cytoskeleton disorganization could both be  
related to a major decrease obsd. in phosphatidylinositol  
4,5-bisphosphate (PtdIns(4,5)P2). Likely in a relationship with  
this decrease, recombinant ADP-ribosylation factor, RhoA, Rac, and  
Rala were not able to reconstitute PLD activity in Lt-treated cells  
permeabilized and depleted of cytosol. Studies of phosphoinositide  
kinase activities did not allow us to attribute the decrease in  
PtdIns(4,5)P2 to inactivation of PtdIns4P 5-kinase. LT was also  
found to provoke a major inhibition in phosphatidylinositol 3-kinase

Searcher : Shears 308-4994

09/126816

that could not account for the inhibition of PLD activity because wortmannin, at doses that fully inhibit phosphatidylinositol 3-kinase, had no effect on the phospholipase activity. Among the three small G-proteins, Ras, Rac, and RalA, inactivated by LT and involved in PLD regulation, inactivation of Ral proteins appeared to be responsible for PLD inhibition as LT toxin (strain 9048) unable to glucosylate Ral proteins did not modify PLD activity. In HL-60 cells, LT treatment appeared also to modify cytosol components in relationship with PLD inhibition as a cytosol prep. from LT-treated cells was less efficient than one from control HL-60 cells in stimulating PLD activity.

Phosphatidylinositol transfer proteins involved in the regulation of polyphosphoinositides and ADP-ribosylation factor, a major cytosolic PLD activator in HL-60 cells, were unchanged, whereas the level of cytosolic protein kinase C. alpha. was decreased after LT treatment. We conclude that in HL-60 cells, lethal toxin from *C. sordellii*, in inactivating small G-proteins involved in PLD regulation, provokes major modifications at the membrane and the cytosol levels that participate in the inhibition of PLD activity. Although Ral appeared to play an essential role in PLD activity, we discuss the role of other small G-proteins inactivated by LT in the different modifications obsd. in HL-60 cells.

L17 ANSWER 2 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2  
ACCESSION NUMBER: 1999:265521 CAPLUS  
DOCUMENT NUMBER: 131:40797  
TITLE: A novel cytotoxin from *Clostridium difficile* serogroup F is a functional hybrid between two other large clostridial cytotoxins  
AUTHOR(S): Chaves-Olarte, Esteban; Low, Peter; Freer, Enrique; Norlin, Thomas; Weidmann, Manfred; Von Eichel-Streiber, Christoph; Thelestam, Monica  
CORPORATE SOURCE: Microbiology and Tumorbiology Center, Karolinska Institutet, Stockholm, S-171 77, Swed.  
SOURCE: J. Biol. Chem. (1999), 274(16), 11046-11052  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The large clostridial cytotoxins (LCTs) constitute a group of high mol. wt. clostridial cytotoxins that inactivate cellular small GTP-binding proteins. We demonstrate that a novel LCT (TcdB-1470) from *Clostridium difficile* strain 1470 is a functional hybrid between "ref." TcdB-10463 and *Clostridium sordellii* TcsL-1522. It bound to the same specific receptor as TcdB-10463 but glucosylated the same GTP-binding proteins as TcsL-1522. All three toxins had equal enzymic potencies but were equally cytotoxic only when

Searcher : Shears 308-4994

09/126816

microinjected. When applied extracellularly TcdB-1470 and TcdB-10463 were considerably more potent cytotoxins than TcsL-1522. The small GTP-binding protein R-Ras was identified as a target for TcdB-1470 and also for TcsL-1522 but not for TcdB-10463. R-Ras is known to control integrin-extracellular matrix interactions from inside the cell. Its glucosylation may be a major determinant for the cell rounding and detachment induced by the two R-Ras-attacking toxins. In contrast, fibroblasts treated with TcdB-10463 were arborized and remained attached, with phosphotyrosine containing structures located at the cell-to-cell contacts and  $\beta$ -integrin remaining at the tips of cellular protrusions. These components were absent from cells treated with the R-Ras-inactivating toxins. The novel hybrid toxin will broaden the utility of the LCTs for clarifying the functions of several small GTPases, now including also R-Ras.

L17 ANSWER 3 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 3  
ACCESSION NUMBER: 1999:350413 CAPLUS  
DOCUMENT NUMBER: 131:142850  
TITLE: Effects of cytotoxic necrotizing factor 1 and lethal toxin on actin cytoskeleton and VE-cadherin localization in human endothelial cell monolayers  
AUTHOR (S): Vouret-Craviari, Valerie; Grall, Dominique; Flatau, Gilles; Pouyssegur, Jacques; Boquet, Patrice; Van Obberghen-Schilling, Ellen  
CORPORATE SOURCE: Centre de Biochimie, CNRS UMR 6543, Nice, 06108, Fr.  
SOURCE: Infect. Immun. (1999), 67(6), 3002-3008  
CODEN: INFIBR; ISSN: 0019-9567  
PUBLISHER: American Society for Microbiology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Integrity of the vascular endothelium is largely dependent on endothelial cell shape and establishment of intercellular junctions. Certain pathogenic bacterial toxins alter the cytoskeletal architecture of intoxicated cells by modulating the GTPase activity of p21 Rho family proteins. In the present study, the authors have analyzed the effect of Rho-directed toxins on the actin cytoskeleton and monolayer integrity of endothelial cells. *Escherichia coli* cytotoxic necrotizing factor 1 (CNF1) activated Rho in human umbilical vein endothelial cells (HUVEC). In confluent monolayers, CNF1 treatment induced prominent stress fiber formation without modifying peripheral localization of VE-cadherin, a specific marker of vascular endothelial cell adherens junctions. Further, Rho activation with CNF1 blocked thrombin-induced redistribution of VE-cadherin staining and gap formation in HUVEC monolayers. Inhibition of Rho by prolonged treatment of cells with C3 exoenzyme

Searcher : Shears 308-4994

09/126816

(*Clostridium botulinum*) eliminated actin stress fibers without disrupting the continuity of VE-cadherin staining, indicating that Rho-dependent stress fibers are not required for maintaining this adhesion receptor at sites of intercellular contact. Lethal toxin (*Clostridium sordellii*), an inhibitor of Rac as well as **Ras** and Rap, potently disrupted the actin microfilament system and monolayer integrity in HUVEC cultures.

L17 ANSWER 4 OF 24 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1999:658079 CAPLUS  
TITLE: The **Ras** superfamily of small  
GTP-binding proteins as targets for bacterial  
toxins  
AUTHOR(S): Boquet, Patrice  
CORPORATE SOURCE: INSERM U452-Faculte de Medecine, Nice, 06107,  
Fr.  
SOURCE: Compr. Sourceb. Bact. Protein Toxins (2nd Ed.)  
(1999), 27-44. Editor(s): Alouf, Joseph E.;  
Freer, John H. Academic: London, UK.  
CODEN: 68GNAN  
DOCUMENT TYPE: Conference  
LANGUAGE: English  
AB Unavailable

L17 ANSWER 5 OF 24 LIFESCI COPYRIGHT 1999 CSA  
ACCESSION NUMBER: 1999:21581 LIFESCI  
TITLE: UDP-glucose Deficiency Causes Hypersensitivity to the  
Cytotoxic Effect of *Clostridium perfringens*  
Phospholipase C  
AUTHOR: Diaz, M.F.; Giron, A.A.; Titball, R.W.; Moos, M.;  
Guillouard, I.; Cole, S.; Howells, A.M.;  
Streiber, C.v.E.; Florin, I.; Thelestam,  
M.  
CORPORATE SOURCE: Microbiology and Tumorbiology Center Karolinska  
Institutet, S-171 77 Stockholm, Sweden  
SOURCE: Journal of Biological Chemistry, (19980918) vol. 273,  
no. 38, pp. 24433-24438.  
ISSN: 0021-9258.  
DOCUMENT TYPE: Journal  
FILE SEGMENT: J  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB A Chinese hamster cell line with a mutation in the UDP-glucose  
pyrophosphorylase (UDPG:PP) gene leading to UDP-glucose deficiency  
as well as a revertant cell were previously isolated. We now show  
that the mutant cell is 10 super(5) times more sensitive to the  
cytotoxic effect of *Clostridium perfringens* phospholipase C (PLC)  
than the revertant cell. To clarify whether there is a connection  
between the UDP-glucose deficiency and the hypersensitivity to C.

Searcher : Shears 308-4994

09/126816

*C. perfringens* PLC, stable transfectant cells were prepared using a wild type UDPG:PP cDNA. Clones of the mutant transfected with a construct having the insert in the sense orientation had increased their UDP-glucose level, whereas those of the revertant transfected with a UDPG:PP antisense had reduced their level of UDP-glucose compared with control clones transfected with the vector. Exposure of these two types of transfectant clones to *C. perfringens* PLC demonstrated that a cellular UDP-glucose deficiency causes hypersensitivity to the cytotoxic effect of this phospholipase. Further experiments with genetically engineered *C. perfringens* PLC variants showed that the sphingomyelinase activity and the C-domain are required for its cytotoxic effect in UDP-glucose-deficient cells.

L17 ANSWER 6 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 4  
ACCESSION NUMBER: 1998:271846 CAPLUS  
DOCUMENT NUMBER: 129:52440  
TITLE: Rho protein inhibition blocks protein kinase C translocation and activation  
AUTHOR(S): Hippensiel, Stefan; Kratz, Thomas; Krull, Matthias; Seybold, Joachim; Eichel-Streiber, Christoph V.; Suttorp, Norbert  
CORPORATE SOURCE: Department of Internal Medicine, Justus-Liebig-University, Giessen, D-35392, Germany  
SOURCE: Biochem. Biophys. Res. Commun. (1998), 245(3), 830-834  
CODEN: BBRCA9; ISSN: 0006-291X  
PUBLISHER: Academic Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Small GTP-binding proteins of the **Ras** and Rho family participate in various important signalling pathways. Large clostridial cytotoxins inactivate GTPases by UDP-glucosylation. Using *Clostridium difficile* toxin B-10463 (TcdB) for inactivation of Rho proteins (RhoA/Rac/Cdc42) and *Clostridium sordellii* lethal toxin-1522 (TcsL) for inactivation of **Ras**-proteins (**Ras**/Rac/Ral, Rap) the role of these GTPases in protein kinase C (PKC) stimulation was studied. Phorbol-myristate-acetate (PMA) induced a rapid PKC translocation to and activation in the particulate cell fraction as detd. by PKC-activity measurements and Western blots for PKC. $\alpha$ . These effects were blocked by TcdB inhibiting Rho proteins in endothelial cells, but not in TcsL-treated cells (i.e., cells without **Ras** activity), suggesting that Rho GTPases (RhoA and/or Cdc42) are the most likely GTP-binding proteins responsible for PKC activation. The Rho requirement for PKC activation/translocation was also verified for human epithelial cells and for lipopolysaccharide-stimulated

Searcher : Shears 308-4994

09/126816

endothelial cells. In summary, the data presented indicate that Rho protein inhibition blocked PKC translocation/activation in endothelial and epithelial cells.

L17 ANSWER 7 OF 24 SCISEARCH COPYRIGHT 1999 ISI (R)  
ACCESSION NUMBER: 1998:891246 SCISEARCH  
THE GENUINE ARTICLE: 136CD  
TITLE: Inhibition of p38 and p42/p44 MAPK by Clostridium sordellii lethal toxin in IL-1 stimulated T lymphocytes - A role for a Ras subfamily G protein in IL-1 signalling  
AUTHOR: Palsson E M (Reprint); Popoff M R; Thelestam M; O'Neill L A J  
CORPORATE SOURCE: TRINITY COLL DUBLIN, DEPT BIOCHEM, DUBLIN, IRELAND; INST PASTEUR, F-75724 PARIS, FRANCE; KAROLINSKA INST, S-17111 STOCKHOLM, SWEDEN  
COUNTRY OF AUTHOR: IRELAND; FRANCE; SWEDEN  
SOURCE: EUROPEAN CYTOKINE NETWORK, (SEP 1998) Vol. 9, No. 3, pp. 129-129.  
Publisher: JOHN LIBBEY EUROTEXT LTD, 127 AVE DE LA REPUBLIQUE, 92120 MONTROUGE, FRANCE.  
ISSN: 1148-5493.  
DOCUMENT TYPE: Conference; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 0

L17 ANSWER 8 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 5  
ACCESSION NUMBER: 1997:533546 CAPLUS  
DOCUMENT NUMBER: 127:195467  
TITLE: Immunotoxin inactivation of Ras subfamily proteins and agents therefor  
INVENTOR(S): Von Eichel-Streiber, Christoph; Boquet, Patrice; Thelestam, Monica  
PATENT ASSIGNEE(S): Boehringer Mannheim G.m.b.H., Germany; Von Eichel-Streiber, Christoph; Boquet, Patrice; Thelestam, Monica  
SOURCE: PCT Int. Appl., 45 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

| PATENT NO. | KIND  | DATE     | APPLICATION NO. | DATE     |
|------------|---|----------|-----------------|----------|
| WO 9727871 | A1  | 19970807 | WO 1997-EP426   | 19970131 |
| W:         | AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, |          |                 |          |
|            | Searcher  | :        | Shears          | 308-4994 |

09/126816

KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO,  
NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA,  
UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB,  
GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA,  
GN, ML, MR, NE, SN, TD, TG

AU 9715982 A1 19970822 AU 1997-15982 19970131  
EP 877622 A1 19981118 EP 1997-902278 19970131

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC,  
PT, IE, FI

PRIORITY APPLN. INFO.: EP 1996-101469 19960202  
WO 1997-EP426 19970131

AB The invention comprises a method of treating a patient with a disorder, characterized by an activating mutation in the **Ras** proto-oncogene, comprising contacting cells of said patient with a protein having the toxic activity of *Clostridium sordellii* toxin LT under conditions favoring inactivating of **Ras** by glucosylation of **Ras**' threonine 35 in said cell. Said protein preferably is an immunotoxin which contains as a toxic domain the catalytic domain of toxin LT.

L17 ANSWER 9 OF 24 TOXLIT  
ACCESSION NUMBER: 1997:129518 TOXLIT  
DOCUMENT NUMBER: CA-127-195467D  
TITLE: Immunotoxin inactivation of **Ras** subfamily proteins and agents therefor.  
AUTHOR: Von Eichel-Streiber C; Boquet P;  
Thelestam M  
SOURCE: (1997). PCT Int. Appl. PATENT NO. 9727871 08/07/1997  
(Thelestam, Monica).  
CODEN: PIXXD2.  
PUB. COUNTRY: GERMANY, FEDERAL REPUBLIC OF  
DOCUMENT TYPE: Patent  
FILE SEGMENT: CA  
LANGUAGE: English  
OTHER SOURCE: CA 127:195467  
ENTRY MONTH: 199805

AB The invention comprises a method of treating a patient with a disorder, characterized by an activating mutation in the **Ras** proto-oncogene, comprising contacting cells of said patient with a protein having the toxic activity of *Clostridium sordellii* toxin LT under conditions favoring inactivating of **Ras** by glucosylation of **Ras**' threonine 35 in said cell. Said protein preferably is an immunotoxin which contains as a toxic domain the catalytic domain of toxin LT.

L17 ANSWER 10 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 6  
ACCESSION NUMBER: 1997:510330 CAPLUS  
DOCUMENT NUMBER: 127:172444  
Searcher : Shears 308-4994

09/126816

TITLE: Escherichia coli cytotoxic necrotizing factor 1 (CNF1), a toxin that activates the Rho GTPase  
AUTHOR(S): Fiorentini, Carla; Fabbri, Alessia; Flatau, Gilles; Donelli, Gianfranco; Matarrese, Paola; Lemichez, Emmanuel; Falzano, Loredana; Boquet, Patrice  
CORPORATE SOURCE: Dep. Ultrastructures, Inst. Superiore Sanita, Rome, 00161, Italy  
SOURCE: J. Biol. Chem. (1997), 272(31), 19532-19537  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Cytotoxic necrotizing factor 1 (CNF1), a 110-kDa protein toxin from pathogenic Escherichia coli induces actin reorganization into stress fibers and retraction fibers in human epithelial cultured cells allowing them to spread. CNF1 is acting in the cytosol since microinjection of the toxin into HEp-2 cells mimics the effects of the externally applied CNF1. Incubation in vitro of CNF1 with recombinant small GTPases induces a modification of Rho (but not of Rac, Cdc42, Ras, or Rab6) as demonstrated by a discrete increase in the apparent mol. wt. of the mol. Preincubation of cells with CNF1 impairs the cytotoxic effects of Clostridium difficult toxin B, which inactivates Rho but not those of Clostridium sordellii LT toxin, which inhibits Ras and Rac. As shown for Rho-GTP, CNF1 activates, in a time- and dose-dependent manner, a cytoskeleton-assocd. phosphatidylinositol 4-phosphate 5-kinase. However, neither the phosphatidylinositol 4,5-bisphosphate (PI 3,4-P2) or 3,4,5-trisphosphate (PIP3) cellular content were found increased in CNF1 treated HEp-2 cells. Cellular effects of CNF1 were not blocked by LY294002, a stable inhibitor of the phosphoinositide 3-kinase. Incubation of HEp-2 cells with CNF1 induces relocalization of myosin 2 in stress fibers but not in retraction fibers. Altogether, our data indicate that CNF1 is a toxin that selectively activates the Rho GTP-binding protein, thus inducing contractility and cell spreading.

L17 ANSWER 11 OF 24 MEDLINE  
DUPLICATE 7  
ACCESSION NUMBER: 97459997 MEDLINE  
DOCUMENT NUMBER: 97459997  
TITLE: Toxins A and B from Clostridium difficile differ with respect to enzymatic potencies, cellular substrate specificities, and surface binding to cultured cells.  
AUTHOR: Chaves-Olarte E; Weidmann M; Eichel-Streiber C; Thelestam M  
CORPORATE SOURCE: Microbiology and Tumorbiology Center (MTC), Karolinska institutet, S-171 77 Stockholm, Sweden.  
SOURCE: JOURNAL OF CLINICAL INVESTIGATION, (1997 Oct 1) 100  
Searcher : Shears 308-4994

09/126816

(7) 1734-41.

Journal code: HS7. ISSN: 0021-9738.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals;  
Cancer Journals

ENTRY MONTH: 199801

ENTRY WEEK: 19980104

AB Clostridium difficile toxins A and B together are responsible for the symptoms of pseudomembranous colitis. Both toxins intoxicate cultured cells by the same mechanism but they differ in cytotoxic potency, toxin B being generally 1,000 times more potent than toxin A. Don and T84 cells were used to determine differences in the intoxication process exerted by both toxins. Three main differences were identified: (a) the specific binding of radiolabeled toxins to the cell surfaces correlated with the cytotoxic potency, (b) toxin B was found to have a 100-fold higher enzymatic activity than toxin A, and (c) toxin A was found to modify an additional substrate, Rap. The relative contribution of (a) and (b) to the difference in cytotoxic potency was determined by microinjection of the toxins. The differing enzymatic activities turned out to be the main determinant of the difference in cytotoxic potency, whereas the difference in binding contributes to a lesser degree. These findings are discussed in the context of the pathophysiological role of the toxins.

L17 ANSWER 12 OF 24 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 97324529 EMBASE

DOCUMENT NUMBER: 1997324529

TITLE: [The action of active bacterial toxins on p21 Rho G proteins: Its role in cellular regulation].  
L'ACTION DES TOXINES BACTERIENNES AGISSANT SUR LA G-PROTEINE P21 RHO: SON ROLE DANS LA REGULATION CELLULAIRE.

AUTHOR: Boquet P.; Gauthier M.

CORPORATE SOURCE: P. Boquet, Unite Inserm U452, Faculte de Medecine, Avenue de Valombrose, 06100 Nice Cedex 2, France.  
u452@unice.fr

SOURCE: Annales de l'Institut Pasteur/Actualites, (1997) 8/2 (173-179).

Refs: 57

ISSN: 0924-4204 CODEN: AIPAEZ

COUNTRY: France

DOCUMENT TYPE: Journal; General Review

FILE SEGMENT: 004 Microbiology

048 Gastroenterology

LANGUAGE: French

SUMMARY LANGUAGE: French

Searcher : Shears 308-4994

09/126816

L17 ANSWER 13 OF 24 BIOSIS COPYRIGHT 1999 BIOSIS  
ACCESSION NUMBER: 1997:333400 BIOSIS  
DOCUMENT NUMBER: PREV199799632603  
TITLE: A mutant cell resistant to *Clostridium difficile* toxins has a low cytosolic level of UDP-glucose.  
AUTHOR(S): Chaves-Olarte, E. (1); Florin, I. (1); Boquet, P.; Von Eichel-Streiber, C.; Thelestam, M.  
(1)  
CORPORATE SOURCE: (1) Microbiol. Tumorbiol. Cent., Karolinska Inst., Box 280, S-171 77 Stockholm Sweden  
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1996) Vol. 28, No. 0, pp. 210-211.  
Meeting Info.: Seventh European Workshop on Bacterial Protein Toxins Hindsgavl, Denmark July 2-7, 1995  
ISSN: 0941-018X.  
DOCUMENT TYPE: Book; Conference  
LANGUAGE: English

L17 ANSWER 14 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 8  
ACCESSION NUMBER: 1996:256012 CAPLUS  
DOCUMENT NUMBER: 124:309937  
TITLE: *Ras*, Rap, and Rac small GTP-binding proteins are targets for *Clostridium sordellii* lethal toxin glucosylation  
AUTHOR(S): Popoff, Michel R.; Chaves-Olarte, Esteban; Lemichez, Emmanuel; von Eichel-Streiber, Christoph; Thelestam, Monica; Chardin, Pierre; Cussac, Didier; Antonny, Bruno; Chavrier, Philippe; et al.  
CORPORATE SOURCE: Inst. Pasteur, Unite Toxines Microbiennes, Paris, 75724, Fr.  
SOURCE: J. Biol. Chem. (1996), 271(17), 10217-24  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Lethal toxin (LT) from *Clostridium sordellii* is one of the high mol. mass clostridial cytotoxins. On cultured cells, it causes a rounding of cell bodies and a disruption of actin stress fibers. We demonstrate that LT is a glucosyltransferase that uses UDP-Glc as a cofactor to covalently modify 21-kDa proteins both in vitro and in vivo. LT glucosylates *Ras*, Rap, and Rac. In *Ras*, threonine at position 35 was identified as the target amino acid glucosylated by LT. Other related members of the *Ras* GTPase superfamily, including RhoA, Cdc42, and Rab6, were not modified by LT. Incubation of serum-starved Swiss 3T3 cells with LT prevents the epidermal growth factor-induced phosphorylation of mitogen-activated protein kinases ERK1 and ERK2, indicating that the toxin blocks *Ras* function in vivo. We also demonstrate

Searcher : Shears 308-4994

09/126816

that LT acts inside the cell and that the glucosylation reaction is required to observe its dramatic effect on cell morphol. LT is thus a powerful tool to inhibit Ras function in vivo.

L17 ANSWER 15 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 9  
ACCESSION NUMBER: 1996:188145 CAPLUS  
DOCUMENT NUMBER: 124:223328  
TITLE: UDP-glucose deficiency in a mutant cell line  
protects against glucosyltransferase toxins from  
Clostridium difficile and Clostridium sordellii  
AUTHOR(S): Chaves-Olarte, Esteban; Florin, Inger;  
Boquet, Patrice; Popoff, Michel; von  
Eichel-Streiber, Christoph; Thelestam,  
Monica  
CORPORATE SOURCE: Microbiology & Tumorbiology Center (MTC),  
Karolinska Inst., Stockholm, S-171 77, Swed.  
SOURCE: J. Biol. Chem. (1996), 271(12), 6925-32  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The authors have previously isolated a fibroblast mutant cell with high resistance to the two Rho-modifying glucosyl-transferase toxins A and B of Clostridium difficile. The authors demonstrate here a low level of UDP-glucose in the mutant, which explains its toxin resistance since: (i) to obtain a detectable toxin B-mediated Rho modification in lysates of mutant cells, addn. of UDP-glucose was required, and it promoted the Rho modification dose-dependently; (ii) high pressure liq. chromatog. anal. of nucleotide exts. of cells indicated that the level of UDP-glucose in the mutant (0.8 nmol/10<sup>6</sup> cells) was lower than in the wild type (3.7 nmol/10<sup>6</sup> cells); and (iii) sensitivity to toxin B was restored upon microinjection of UDP-glucose. Using the mutant as indicator cell the authors also found that the related Clostridium sordellii lethal toxin is a glucosyltransferase which requires UDP-glucose as a cofactor. Like toxin B it glucosylated 21-23-kDa proteins in cell lysates, but Rho was not a substrate for lethal toxin.

L17 ANSWER 16 OF 24 MEDLINE DUPLICATE 10  
ACCESSION NUMBER: 97055675 MEDLINE  
DOCUMENT NUMBER: 97055675  
TITLE: Large clostridial cytotoxins--a family of  
glycosyltransferases modifying small GTP-binding  
proteins.  
AUTHOR: von Eichel-Streiber C; Boquet P; Sauerborn  
M; Thelestam M  
CORPORATE SOURCE: Institut fur Medizinische Mikrobiologie und Hygiene,  
Johannes Gutenberg-Universitdt Mainz, Germany..  
veichel@goofy.zdv.uni.mainz.de  
SOURCE: TRENDS IN MICROBIOLOGY, (1996 Oct) 4 (10) 375-82.  
Searcher : Shears 308-4994

09/126816

Ref: 55  
Journal code: B1N. ISSN: 0966-842X.  
PUB. COUNTRY: ENGLAND: United Kingdom  
Journal; Article; (JOURNAL ARTICLE)  
General Review; (REVIEW)  
(REVIEW, TUTORIAL)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199704  
ENTRY WEEK: 19970402  
AB Some Clostridium species produce ABX-type protein cytotoxins of high molecular weight. These toxins constitute the group of large clostridial cytotoxins (LCTs), which have homologous protein sequences, exert glycosyltransferase activity and modify GTP-binding proteins of the Ras-superfamily. These characteristics render the LCTs valuable tools for developmental and cell biologists.

L17 ANSWER 17 OF 24 TOXLINE  
ACCESSION NUMBER: 1997:121090 TOXLINE  
DOCUMENT NUMBER: BIOSIS-97-22511  
TITLE: A MUTANT CELL RESISTANT TO CLOSTRIDIUM DIFFICILE TOXINS HAS A LOW CYTOSOLIC LEVEL OF UDP-GLUCOSE.  
AUTHOR: CHAVES-OLARTE E; FLORIN I; BOQUET P; VON EICHEL-STREIBER C; THELESTAM M  
SOURCE: SEVENTH EUROPEAN WORKSHOP ON BACTERIAL PROTEIN TOXINS, HINDSGAVL, DENMARK, JULY 2-7, 1995.  
ZENTRALBLATT FUER BAKTERIOLOGIE SUPPLEMENT, (1996).  
Vol. 28, pp. 210-211.  
CODEN: ZBASE2.  
FILE SEGMENT: BIOSIS  
LANGUAGE: English  
ENTRY MONTH: 199709  
AB BIOSIS COPYRIGHT: BIOL ABS. RRM BOOK CHAPTER MEETING PAPER  
CLOSTRIDIUM-DIFFICILE ANIMAL ENZYMOLOGY TOXICOLOGY UDP-GLUCOSE  
CYTOSOL LOCALIZATION TOXIN A TOXIN B RESISTANCE GLUCOSYL TRANSFERASE  
RHO SMALL GTPASE ADP-RIBOSYLATION FIBROBLAST CELL BIOLOGY CYTOSOL

L17 ANSWER 18 OF 24 SCISEARCH COPYRIGHT 1999 ISI (R)  
ACCESSION NUMBER: 94:203599 SCISEARCH  
THE GENUINE ARTICLE: NF017  
TITLE: CLOSTRIDIUM-DIFFICILE TOXIN-B ACTS ON THE GTP-BINDING PROTEIN-RHO  
AUTHOR: JUST I (Reprint); FRITZ G; AKTORIES K; GIRY M;  
POPOFF M R; BOQUET P; HEGENBARTH S;  
VONEICHELSTREIBER C  
CORPORATE SOURCE: UNIV SAARLAND, INST PHARMACOL & TOXIKOL, D-66421  
HOMBURG, GERMANY (Reprint); INST PASTEUR, UNITE  
TOXINES BACTERIENNES, F-75724 PARIS 15, FRANCE; UNIV  
Searcher : Shears 308-4994

09/126816

COUNTRY OF AUTHOR: MAINZ, INST MED MICROBIOL, D-55101 MAINZ, GERMANY  
GERMANY; FRANCE  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (08 APR 1994) Vol.  
269, No. 14, pp. 10706-10712.  
ISSN: 0021-9258.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 48

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Clostridium difficile toxin B exhibits cytotoxic activity that is characterized by the disruption of the microfilamental cytoskeleton. Here we studied whether the GTP-binding Rho protein, which reportedly participates in the regulation of the actin cytoskeleton, is involved in the toxin action. Toxin B treatment of Chinese hamster ovary cells reveals a time- and concentration-dependent decrease in the ADP-ribosylation of Rho by Clostridium botulinum C3 exoenzyme in the cell lysate. Disruption of the microfilament system induced by C. botulinum C2 toxin or cytochalasin D does not cause impaired ADP-ribosylation of Rho. Toxin B exhibits its effects on Rho not only in intact cells but also when added to cell lysates. Besides endogenous Rho, RhoA-glutathione S-transferase (Rho-GST) fusion protein added to cell lysate showed decreased ADP-ribosylation after toxin B treatment. Immunoblot analysis reveals identical amounts of Rho-GST and no change in molecular mass after toxin B treatment compared with controls. ADP-ribosylation of Rho-GST purified from toxin B-treated cell lysate is inhibited, indicating a modification of Rho itself. Finally, transfection of rhoA DNA under the control of a strong promoter into cells protects them from the activity of toxin B. Altogether, the data indicate that C. difficile toxin B acts directly or indirectly on Rho proteins to inhibit ADP-ribosylation and suggest that the cytotoxic effect of toxin B involves Rho.

L17 ANSWER 19 OF 24 SCISEARCH COPYRIGHT 1999 ISI (R)  
ACCESSION NUMBER: 94:253931 SCISEARCH  
THE GENUINE ARTICLE: NJ034  
TITLE: CYTOTOXIC NECROTIZING FACTOR TYPE-2 PRODUCED BY  
VIRULENT ESCHERICHIA-COLI MODIFIES THE SMALL  
GTP-BINDING PROTEINS-RHO INVOLVED IN ASSEMBLY OF  
ACTIN STRESS FIBERS  
AUTHOR: OSWALD E; SUGAI M; LABIGNE A; WU H C; FIORENTINI C;  
BOQUET P; O'BRIEN A D (Reprint)  
CORPORATE SOURCE: UNIFORMED SERV UNIV HLTH SCI, DEPT MICROBIOL &  
IMMUNOL, BETHESDA, MD, 20814 (Reprint); UNIFORMED  
SERV UNIV HLTH SCI, DEPT MICROBIOL & IMMUNOL,  
BETHESDA, MD, 20814; HIROSHIMA UNIV, SCH DENT, DEPT  
MICROBIOL, HIROSHIMA 734, JAPAN; INST PASTEUR,  
INSERM, U193, UNITE ENTEROBACTERIES, F-75015 PARIS,  
Searcher : Shears 308-4994

09/126816

other cells rapidly underwent temporary morphol. alterations that were in certain respects similar to those seen after microinjection of cloned gene ras proteins. When injected into Xenopus oocytes, C3 induced migration of germinal vesicles and potentiated the cholera toxin-sensitive augmentation of germinal vesicle breakdown by progesterone, also as caused by ras proteins. Nevertheless, p21.bot was immunol. distinct from p21ras.

L17 ANSWER 24 OF 24 LIFESCI COPYRIGHT 1999 CSA  
ACCESSION NUMBER: 86:71890 LIFESCI  
TITLE: Structure/function relationships of tetanus toxin. A comparison with the diphtheria toxin molecule. BACTERIAL PROTEIN TOXINS.  
AUTHOR: Roa, M.; Kagan, B.L.; Boquet, P.; Falmagne, P. [editor]; Alouf, J.E. [editor]; Fehrenbach, F.J. [editor]; Jeljaszewicz, J. [editor]; Thelestam, M. [editor]  
CORPORATE SOURCE: Unite Antigenes Bact. (UA CNRS 040557), Inst. Pasteur, 75724 Paris Cedex 15, France  
SOURCE: ZENTRALBL. BAKTERIOL. MIKROBIOL. HYG., (1986) pp. 27-32.  
Meeting Info.: 2. European Workshop on Bacterial Protein Toxins. Wepion (Belgium). 30 Jun-4 Jul 1985.  
ISBN: 3-437-11083-7.  
DOCUMENT TYPE: Book  
TREATMENT CODE: Conference  
FILE SEGMENT: J; X  
LANGUAGE: English

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, LIFESCI, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, PROMT, TOXLIT, TOXLINE, CANCERLIT, USPATFULL' ENTERED AT 11:46:17 ON 16 NOV 1999)

L18 400 S VON EICHEL ?/AU  
L19 109 S L18 AND (RAS OR L10 OR L11)  
L20 76 S L19 NOT L16  
L21 18 DUP REM L20 (58 DUPLICATES REMOVED)

L21 ANSWER 1 OF 18 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1999:658085 CAPLUS  
TITLE: Clostridial toxins acting on the cytoskeleton  
AUTHOR(S): Thelestam, Monica; Chaves-Olarde, Esteban; Moos, Michael; Von Eichel-Streiber, Christoph  
CORPORATE SOURCE: Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm, S-17177, Swed.  
SOURCE: Compr. Sourceb. Bact. Protein Toxins (2nd Ed.) (1999), 147-173. Editor(s): Alouf, Joseph E.; Freer, John H. Academic: London, UK.  
CODEN: 68GNAV  
Searcher : Shears 308-4994

09/126816

DOCUMENT TYPE: Conference  
LANGUAGE: English  
AB Unavailable

L21 ANSWER 2 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1  
ACCESSION NUMBER: 1999:486236 CAPLUS  
DOCUMENT NUMBER: 131:211505  
TITLE: The actin-based motility of intracellular Listeria monocytogenes is not controlled by small GTP-binding proteins of the rho- and Ras-subfamilies  
AUTHOR(S): Ebel, Frank; Rohde, Manfred; Von Eichel-Streiber, Christoph; Wehland, Jurgen; Chakraborty, Trinad  
CORPORATE SOURCE: Institut fur Medizinische Mikrobiologie, Justus-Liebig-Universitat, Giessen, 35392, Germany  
SOURCE: FEMS Microbiol. Lett. (1999), 176(1), 117-124  
CODEN: FMLED7; ISSN: 0378-1097  
PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB In this study, the authors analyzed whether the actin-based motility of intracellular Listeria monocytogenes is controlled by the small GTP-binding proteins of the Rho- and Ras-subfamilies. These signalling proteins are key regulatory elements in the control of actin dynamics and their activity is essential for the maintenance of most cellular microfilament structures. The authors used the Clostridium difficile toxins TcdB-10463 and TcdB-1470 to specifically inactivate these GTP-binding proteins. Treatment of eukaryotic cells with either of these toxins led to a dramatic breakdown of the normal actin cytoskeleton, but did not abrogate the invasion of epithelial cells by L. monocytogenes and had no effect on the actin-based motility of this bacterial parasite. The data indicate that intracellular Listeria reorganize the actin cytoskeleton in a way that circumvents the control mechanisms mediated by the members of the Rho- and Ras-subfamilies that can be inactivated by the TcdB-10463 and TcdB-1470 toxins.

L21 ANSWER 3 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2  
ACCESSION NUMBER: 1998:638922 CAPLUS  
DOCUMENT NUMBER: 130:21586  
TITLE: UDP-glucose deficiency causes hypersensitivity to the cytotoxic effect of Clostridium perfringens phospholipase C  
AUTHOR(S): Flores-Diaz, Marietta; Alape-Giron, Alberto; Titball, Richard W.; Moos, Michael; Guillouard, Isabelle; Cole, Stewart; Howells, Angela M.; Von Eichel-Streiber, Christoph; Florin, Searcher : Shears 308-4994

09/126816

CORPORATE SOURCE: Inger, Thelestam, Monica  
Microbiology and Tumorbiology Center, Karolinska  
Institutet, Stockholm, S-171 77, Swed.  
SOURCE: J. Biol. Chem. (1998), 273(38), 24433-24438  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular  
Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB A Chinese hamster cell line with a mutation in the UDP-glucose pyrophosphorylase (UDPG:PP) gene leading to UDP-glucose deficiency as well as a revertant cell were previously isolated. The authors now show that the mutant cell is 105 times more sensitive to the cytotoxic effect of Clostridium perfringens phospholipase C (PLC) than the revertant cell. To clarify whether there is a connection between the UDP-glucose deficiency and the hypersensitivity to C. perfringens PLC, stable transfectant cells were prep'd. using a wild type UDPG:PP cDNA. Clones of the mutant transfected with a construct having the insert in the sense orientation had increased their UDP-glucose level, whereas those of the revertant transfected with a UDPG:PP antisense had reduced their level of UDP-glucose compared with control clones transfected with the vector. Exposure of these two types of transfectant clones to C. perfringens PLC demonstrated that a cellular UDP-glucose deficiency causes hypersensitivity to the cytotoxic effect of this phospholipase. Further expts. with genetically engineered C. perfringens PLC variants showed that the sphingomyelinase activity and the C-domain are required for its cytotoxic effect in UDP-glucose-deficient cells.

L21 ANSWER 4 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 3  
ACCESSION NUMBER: 1998:223590 CAPLUS  
DOCUMENT NUMBER: 128:318211  
TITLE: Specific inhibition of phorbol ester-stimulated phospholipase D by Clostridium sordellii lethal toxin and Clostridium difficile toxin B-1470 in HEK-293 cells. Restoration by Ral GTPases  
AUTHOR(S): Schmidt, Martina; Voss, Matthias; Thiel, Markus; Bauer, Bettina; Grannass, Andreas; Tapp, Eva; Cool, Robbert H.; De Gunzburg, Jean; Von Eichel-Streiber, Christoph; Jakobs, Karl H.  
CORPORATE SOURCE: Universitatsklinikum Essen, Institut fur Pharmakologie, Essen, D-45122, Germany  
SOURCE: J. Biol. Chem. (1998), 273(13), 7413-7422  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

Searcher : Shears 308-4994

09/126816

AB To study whether **Ras**-like GTPases are involved in phospholipase D (PLD) regulation, we studied the effects of the *Clostridium difficile* toxin B (TcdB) variant TcdB-1470 and *Clostridium sordellii* lethal toxin (TcsL), known to inactivate Rac and some members of the **Ras** protein family, on PLD activities. TcdB-1470 and TcsL did not affect basal PLD activity and PLD stimulation by m<sub>3</sub> muscarinic acetylcholine receptor (mAChR) or direct G protein activation. In contrast, PMA-induced PLD stimulation was inhibited by TcdB-1470 and TcsL in a time- and concn.-dependent manner, without alteration in immunol. detectable protein kinase C (PKC) isoenzyme levels. In membranes of HEK-293 cells pretreated with TcdB-1470 or TcsL, basal and stable GTP analog-stimulated PLD activities measured with exogenous phosphatidylcholine, in the presence or absence of phosphatidylinositol 4,5-bisphosphate, were not altered. In contrast, pretreatment with TcdB-1470 and TcsL, but not TcdB, strongly reduced PMA-stimulated PLD activity. The addn. of recombinant Rac1, serving as glucosylation substrate for TcdB, TcsL, and TcdB-1470, did not restore PLD stimulation by PMA. Furthermore, PMA-stimulated PLD activity, suppressed by prior treatment with TcdB-1470 or TcsL, was not rescued by the addn. of recombinant **Ras** (RasG12V) or Rap proteins, acting as glucosylation substrates for TcsL only (**Ras**) or TcdB-1470 and TcsL (Rap). In contrast, the addn. of recombinant Ral proteins (RalA and RalB), glucosylation substrates for TcsL and TcdB-1470, but not for TcdB, to membranes of TcdB-1470- or TcsL-treated cells fully restored PLD stimulation by PMA without altering the strict MgATP dependence of PMA-induced PLD stimulation. RalA-mediated restoration of PMA-stimulated PLD activity in membranes of TcsL-treated cells was not enhanced by coaddn. of RasG12V. In conclusion, the data presented indicate that TcdB-1470 and TcsL selectively interfere with phorbol ester stimulation of PLD and suggest an essential role of Ral proteins in PKC signaling to PLD in HEK-293 cells.

L21 ANSWER 5 OF 18 MEDLINE DUPLICATE 4  
ACCESSION NUMBER: 1998249799 MEDLINE  
DOCUMENT NUMBER: 98249799  
TITLE: Rho protein inhibition blocks protein kinase C translocation and activation.  
AUTHOR: Hippenstiel S; Kratz T; Krull M; Seybold J; von Eichel-Streiber C; Suttorp N  
CORPORATE SOURCE: Department of Internal Medicine, Justus-Liebig-University, Giessen, Germany.  
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Apr 28) 245 (3) 830-4.  
PUB. COUNTRY: United States  
Journal code: 9Y8. ISSN: 0006-291X.  
Journal; Article; (JOURNAL ARTICLE)  
Searcher : Shears 308-4994

09/126816

LANGUAGE: English  
FILE SEGMENT: Priority Journals; Cancer Journals  
ENTRY MONTH: 199808  
ENTRY WEEK: 19980802

AB Small GTP-binding proteins of the **Ras** and Rho family participate in various important signalling pathways. Large clostridial cytotoxins inactivate GTPases by UDP-glucosylation. Using *Clostridium difficile* toxin B-10463 (TcdB) for inactivation of Rho proteins (RhoA/Rac/Cdc42) and *Clostridium sordellii* lethal toxin-1522 (TcsL) for inactivation of **Ras**-proteins (**Ras**/Rac/Ral, Rap) the role of these GTPases in protein kinase C (PKC) stimulation was studied. Phorbol-myristate-acetate (PMA) induced a rapid PKC translocation to and activation in the particulate cell fraction as determined by PKC-activity measurements and Western blots for PKC alpha. These effects were blocked by TcdB inhibiting Rho proteins in endothelial cells, but not in TcsL-treated cells (i.e., cells without **Ras** activity), suggesting that Rho GTPases (RhoA and/or Cdc42) are the most likely GTP-binding proteins responsible for PKC activation. The Rho requirement for PKC activation/translocation was also verified for human epithelial cells and for lipopolysaccharide-stimulated endothelial cells. In summary, the data presented indicate that Rho protein inhibition blocked PKC translocation/activation in endothelial and epithelial cells.

L21 ANSWER 6 OF 18 CAPLUS COPYRIGHT 1999 ACS  
ACCESSION NUMBER: 1999:6411 CAPLUS  
DOCUMENT NUMBER: 130:221101  
TITLE: Activation and inactivation of **ras**-like GTPases by bacterial cytotoxins  
AUTHOR(S): **Von Eichel-Streiber, Christoph**, Weidmann, Manfred; Giry, Murielle; Moos, Michael  
CORPORATE SOURCE: Verfugungsgebäude für Forschung und Entwicklung, Institut für Medizinische Mikrobiologie und Hygiene, Mainz, D-55101, Germany  
SOURCE: Methods Microbiol. (1998), 27 (Bacterial Pathogenesis), 509-525  
CODEN: MMICEU; ISSN: 0580-9517  
PUBLISHER: Academic Press  
DOCUMENT TYPE: Journal; General Review  
LANGUAGE: English

AB A review with 54 refs. Topics include: modulation of the eukaryotic skeleton by bacterial products; cytoskeletal targets of bacterial toxins; the switch characteristic of GTPases; Rho-modulating bacterial toxins; **Ras**-modulating bacterial factors; use of LCTs as tools in cell biol. (c) 1998 Academic Press.

L21 ANSWER 7 OF 18 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 5  
ACCESSION NUMBER: 1998:459143 BIOSIS  
Searcher : Shears 308-4994

09/126816

DOCUMENT NUMBER: PREV199800459143  
TITLE: A UDP-glucose deficient cell mutant as model to study the molecular mechanism of cytotoxicity induced by *C. perfringens* phospholipase C in ischemic tissue.  
AUTHOR(S): Thelestam, M. (1); Flores Dfaz, M. (1); Alape Giron, A. (1); Titball, R.; Pollesello, P.; Persson, B.; Moos, M.; Chaves Olarte, E. (1); Lofrumento, D.; Cortes Bratii, X. (1); Bergman, T.; Von Eichel-Streiber, C.; Florin, I. (1)  
CORPORATE SOURCE: (1) Microbiol. and Tumorbiol. Cent., Karolinska Inst., S-171 77 Stockholm Sweden  
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1998) Vol. 29, pp. 184-191.  
Meeting Info.: Eighth European Workshop on Bacterial Protein Toxins Staffelstein, Kloster Banz, Germany June 29-July 4, 1997  
ISSN: 0941-018X.  
DOCUMENT TYPE: Conference  
LANGUAGE: English

L21 ANSWER 8 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 6  
ACCESSION NUMBER: 1998:397225 CAPLUS  
DOCUMENT NUMBER: 129:134470  
TITLE: Small GTP-binding proteins of the Rho- and Ras-subfamilies are not involved in the actin rearrangements induced by attaching and effacing *Escherichia coli*  
AUTHOR(S): Ebel, Frank; von Eichel-Streiber, Christoph; Rohde, Manfred; Chakraborty, Trinad  
CORPORATE SOURCE: Institut fur Medizinische Mikrobiologie, Justus-Liebig-Universitat, Giessen, D-35392, Germany  
SOURCE: FEMS Microbiol. Lett. (1998), 163(2), 107-112  
CODEN: FMLED7; ISSN: 0378-1097  
PUBLISHER: Elsevier Science B.V.  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Attaching and effacing *Escherichia coli* (AEEC) are extracellular pathogens that induce the formation of actin-rich structures at their sites of attachment to eukaryotic host cells. We analyzed whether small GTP-binding proteins of the Rho- and Ras-subfamilies, which control the cellular actin system, are essential for these bacterial-induced microfilament reorganizations. For this purpose we specifically inactivated them using the *Clostridium difficile* toxins TcdB-10463 and TcdB-1470. Such treatment led to a dramatic breakdown of the normal actin cytoskeleton, but did not abrogate the bacterial-induced actin rearrangements. Our data therefore indicate that the microfilament reorganizations induced by

Searcher : Shears 308-4994

09/126816

AEEC are independent of those small GTP-binding proteins that under normal conditions control the dynamics and maintenance of the actin cytoskeleton.

L21 ANSWER 9 OF 18 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 7  
ACCESSION NUMBER: 1998:459113 BIOSIS  
DOCUMENT NUMBER: PREV199800459113  
TITLE: Toxins A and B from Clostridium difficile differ with respect to enzymatic potencies, cellular substrate specificities and surface binding to cultured cells.  
AUTHOR(S): Chaves-Olarte, E. (1); Weidmann, M.; Von Eichel-Streiber, C.; Thelestam, M. (1)  
CORPORATE SOURCE: (1) Microbiol. and Tumorbiol. Cent., Karolinska Inst., Stockholm Sweden  
SOURCE: Zentralblatt fuer Bakteriologie Supplement, (1998) Vol. 29, pp. 74-75.  
Meeting Info.: Eighth European Workshop on Bacterial Protein Toxins Staffelstein, Kloster Banz, Germany June 29-July 4, 1997  
ISSN: 0941-018X.  
DOCUMENT TYPE: Conference  
LANGUAGE: English

L21 ANSWER 10 OF 18 BIOSIS COPYRIGHT 1999 BIOSIS  
ACCESSION NUMBER: 1998:293569 BIOSIS  
DOCUMENT NUMBER: PREV199800293569  
TITLE: Role of Ral GTPases in protein kinase C-induced phospholipase D stimulation.  
AUTHOR(S): Voss, M. (1); Bauer, B.; Cool, R. H.; Von Eichel-Streiber, C.; Jakobs, K. H. (1); Schmidt, M. (1)  
CORPORATE SOURCE: (1) Inst. Pharmakol., Univ. GH Essen, D-45122 Essen Germany  
SOURCE: Naunyn-Schmiedeberg's Archives of Pharmacology, (1998) Vol. 357, No. 4 SUPPL, pp. R56.  
Meeting Info.: 39th Spring Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology Mainz, Germany March 17-19, 1998  
German Society for Experimental and Clinical Pharmacology and Toxicology  
ISSN: 0028-1298.  
DOCUMENT TYPE: Conference  
LANGUAGE: English

L21 ANSWER 11 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 8  
ACCESSION NUMBER: 1997:637149 CAPLUS  
DOCUMENT NUMBER: 127:329879  
TITLE: Cellular UDP-glucose deficiency caused by a single point mutation in the UDP-glucose Searcher : Shears 308-4994

09/126816

AUTHOR(S) : pyrophosphorylase gene  
Flores-Diaz, Marietta; Alape-Giron, Alberto;  
Persson, Bengt; Pollesello, Piero; Moos,  
Michael; von Eichel-Streiber, Christoph  
; Thelestam, Monica; Florin, Inger  
CORPORATE SOURCE: Microbiology and Tumorbiology Center and Dep. of  
Medical Biochemistry and Biophysics, Karolinska  
Institutet, Stockholm, S-171 77, Swed.  
SOURCE: J. Biol. Chem. (1997), 272(38), 23784-23791  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular  
Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The authors previously isolated a mutant cell that is the only mammalian cell reported to have a persistently low level of UDP-glucose. In this work the authors obtained a spontaneous revertant whose UDP-glucose level lies between those found in the wild type and the mutant cell. The activity of UDP-glucose pyrophosphorylase (UDPG:PP), the enzyme that catalyzes the formation of UDP-glucose, was in the mutant 4% and in the revertant 56% of the activity found in the wild type cell. Sequence anal. of UDPG: PP cDNAs from the mutant cell showed one missense mutation, which changes amino acid residues 115 from glycine to aspartic acid. The substituted glycine is located within the largest stretch of strictly conserved residues among eukaryotic UDPG:PPs. The anal. of the cDNAs from the revertant cell indicated the presence of an equimolar mixt. of the wild type and the mutated mRNAs, suggesting that the mutation has reverted in only one of the alleles. In summary, the authors demonstrate that the G115D substitution in the Chinese hamster UDPG:PP dramatically impairs its enzymic activity, thereby causing cellular UDP-glucose deficiency.

L21 ANSWER 12 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 9  
ACCESSION NUMBER: 1997:653189 CAPLUS  
DOCUMENT NUMBER: 127:274019  
TITLE: Toxins A and B from Clostridium difficile differ with respect to enzymic potencies, cellular substrate specificities, and surface binding to cultured cells  
AUTHOR(S) : Chaves-Olarte, Esteban; Weidmann, Manfred;  
Von Eichel-Streiber, Christoph;  
Thelestam, Monica  
CORPORATE SOURCE: Microbiology and Tumorbiology Center (MTC),  
Karolinska institutet, Stockholm, S-171 77,  
Swed.  
SOURCE: J. Clin. Invest. (1997), 100(7), 1734-1741  
CODEN: JCINAO; ISSN: 0021-9738  
PUBLISHER: Rockefeller University Press  
Searcher : Shears 308-4994

09/126816

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB C. difficile toxins A and B together are responsible for the symptoms of pseudomembranous colitis. Both toxins intoxicate cultured cells by the same mechanism but they differ in cytotoxic potency, toxin B being generally 1000 times more potent than toxin A. Don and T84 cells were used to det. differences in the intoxication process exerted by both toxins. Three main differences were identified: (1) the specific binding of radiolabeled toxins to the cell surfaces correlated with the cytotoxic potency, (2) toxin B was found to have a 100-fold higher enzymic activity than toxin A, and (3) toxin A was found to modify an addnl. substrate, Rap. The relative contribution of (1) and (2) to the difference in cytotoxic potency was detd. by microinjection of the toxins. The differing enzymic activities turned out to be the main determinant of the difference in cytotoxic potency whereas the difference in binding contributes to a lesser degree. These findings are discussed in the context of the pathophysiol. role of the toxins.

L21 ANSWER 13 OF 18 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:419486 CAPLUS

DOCUMENT NUMBER: 129:187684

TITLE: A UDP-glucose deficient cell mutant as model to study the molecular mechanism of cytotoxicity induced by C. perfringens phospholipase C in ischemic tissue

AUTHOR(S): Thelestam, M.; Flores Dfaz, M.; Alape Giron, A.; Titball, R.; Pollesello, P.; Persson, B.; Moos, M.; Chaves Olarte, E.; Lofrumento, D.; Cortes Bratii, X.; Bergman, T.; Von Eichel-Streiber, C.; Florin, I.

CORPORATE SOURCE: Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm, S-171 77, Swed.

SOURCE: Zentralbl. Bakteriol., Suppl. (1997), 29(Bacterial Protein Toxins), 184-191

CODEN: ZBASE2; ISSN: 0941-018X

PUBLISHER: Gustav Fischer Verlag

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A Chinese hamster mutant cell line has a UDPG deficiency conferring resistance to glucosyltransferase toxins. The deficiency is caused by a point mutation in the UDPG:pyrophosphorylase (UDPG:PP) gene, as shown by sequence anal. and confirmed by transfection expts. The mutant cell overproduces calreticulin (CRT) and four glucose-regulated stress proteins (GRPs) and is hypersensitive to C. perfringens phospholipase C (PLC). Wild type cells subjected to glucose starvation exhibit a UDPG deficiency, up-regulate CRT and GRPs and are also hypersensitive to PLC. Transfection of the mutant cell with bovine UDPG:PP cDNA demonstrated a linkage between the

Searcher : Shears 308-4994

09/126816

UDPG deficiency and the hypersensitivity to PLC. The mutant cell will be a useful model to clarify the mol. mechanism of the cytotoxicity induced by PLC as well as the role of UDPG for mol. signaling in cells under glucose starvation.

L21 ANSWER 14 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 10  
ACCESSION NUMBER: 1997:420507 CAPLUS  
DOCUMENT NUMBER: 127:77178  
TITLE: Delineation of the catalytic domain of Clostridium difficile toxin B-10463 to an enzymically active N-terminal 467 amino acid fragment  
AUTHOR(S): Wagenknecht-Wiesner, Alice; Weidmann, Manfred; Braun, Veit; Leukel, Petra; Moos, Michael; von Eichel-Streiber, Christoph  
CORPORATE SOURCE: Verfuegungsgebaeude fuer Forschung und Entwicklung, Institut fuer medizinische Mikrobiologie und Hygiene, Johannes Gutenberg-Universitaet, Obere Zahlbacherstr. 63, Mainz, 55101, Germany  
SOURCE: FEMS Microbiol. Lett. (1997), 152(1), 109-116  
CODEN: FMLED7; ISSN: 0378-1097  
PUBLISHER: Elsevier  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB To directly approach the postulated toxic domain of Clostridium difficile's TcdB-10463, eight subclones of different size and locations in the N-terminal third of the toxin were generated. Expression of these toxin fragments was checked in Western blots and the enzymic activity of the expressed proteins was analyzed by glucosylating Ras related small GTP-binding proteins. Two polypeptides of 875 aa (TcdBc1-3) and 557 aa (TcdBc1-H) glucosylated their targets Rho, Rac and Cdc42 with the same activity and specificity as the holotoxin. In comparison 516 aa (TcdBc1-N) and 467 aa (TcdBc1-A) protein fragments exhibited highly reduced activity, while TcdBc1 and TcdBc2-3 (aa 1-243 and 244-890, resp.) were enzymically inactive. Our results indicate that all structures involved in the catalysis are located at several different sites within the 557 aa fully active fragment. The shortest enzymically still active protein covers aa 1-467 and obviously fulfills all minimal requirements for glucosylation. The data support the postulated three domain model of 'large clostridial cytotoxins'.

L21 ANSWER 15 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 11  
ACCESSION NUMBER: 1995:628937 CAPLUS  
DOCUMENT NUMBER: 123:27527  
TITLE: The enterotoxin from Clostridium difficile (ToxA) monoglycosylates the Rho proteins  
AUTHOR(S): Just, Ingo; Wilm, Matthias; Selzer, Joerg; Rex, Searcher : Shears 308-4994

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CORPORATE SOURCE: Gundula; von Eichel-Streiber, Christoph  
; Mann, Matthias; Aktories, Klaus  
Institut Pharmakologie, Toxikologie, Universitat  
Saarlandes, Hamburg/Saar, D-66421, Germany  
SOURCE: J. Biol. Chem. (1995), 270(23), 13932-6  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Here we report on the identification of the enterotoxin from Clostridium difficile (ToxA)-induced modification of Rho proteins. From several hexoses tested UDP-glucose selectively served as cosubstrate for ToxA-catalyzed modification. The acceptor amino acid of glucosylation was identified from a Lys-C-generated peptide by tandem mass spectrometry as Thr-37. mutation of Thr-37 to Ala completely abolished glucosylation. The members of the Rho family (RhoA, Rac1, and Cdc42Hs) were substrates for ToxA, whereas H-Ras, Rab5, and Arf1 were not glucosylated. ToxA-catalyzed glucosylation of lysates from ToxA-pretreated rat basophilic leukemia (RBL) cells resulted in a decreased incorporation of [14C]glucose, indicating previous glucosylation in the intact cell. Glucosylation of the Rho subtype proteins appears to be the mol. mechanism by which C. difficile ToxA mediates its cytotoxic effects on cells.

L21 ANSWER 16 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 12  
ACCESSION NUMBER: 1995:832244 CAPLUS  
DOCUMENT NUMBER: 123:224757  
TITLE: Transient expression of RhoA, -B, and -C GTPases  
in HeLa cells potentiates resistance to  
Clostridium difficile toxins A and B but not to  
Clostridium sordellii lethal toxin  
AUTHOR(S): Giry, Murielle; Popoff, Michel R.; von  
Eichel-Streiber, Christoph; Boquet,  
Patrice

CORPORATE SOURCE: Unite des Toxines Microbiennes, Institut  
Pasteur, Paris, 75724, Fr.

SOURCE: Infect. Immun. (1995), 63(10), 4063-71  
CODEN: INFIBR; ISSN: 0019-9567

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The bacterial pathogen Clostridium difficile synthesizes two high-mol.-wt. toxins (A and B), which exhibit toxic effects in vivo and in vitro. Here, the authors present evidence that the major intracellular targets of these two toxins are the Rho GTPases. Overexpression of RhoA, RhoB, or RhoC GTPases in transfected HeLa cells conferred an increased resistance to toxins A and B, indicating that these toxins cause their cytopathic effects primarily by affecting Rho proteins. In addn., toxin A and B treatment appeared to result in modification of Rho, since Rho

Searcher : Shears 308-4994

09/126816

isolated from toxin-treated cells had a decreased ability to be ADP-ribosylated by Clostridium botulinum C3 exoenzyme. In contrast, the lethal toxin (LT) of Clostridium sordellii, although structurally and immunol. related to C. difficile toxin B, appeared to induce cytopathic effects independently of the Rho GPTases. Overexpression of RhoA in transfected HeLa cells did not protect them from the effect of LT, and Rho isolated from lysates of LT-treated cells was not resistant to modification by C3. Immunofluorescence studies showed that LT treatment caused a cytopathic effect that was very different from those described for C. difficile toxins A and B, resulting in an increase in cortical F-actin, which a concomitant decrease in the no. of stress fibers, and in the formation of numerous microvilli contg. the actin-bundling protein fimbrin/plastin.

L21 ANSWER 17 OF 18 BIOSIS COPYRIGHT 1999 BIOSIS DUPLICATE 13  
ACCESSION NUMBER: 1996:44030 BIOSIS  
DOCUMENT NUMBER: PREV199698616165  
TITLE: Effects of Clostridium difficile toxins A and B and the lethal toxin of Clostridium sordellii on the Rho GTPases.  
AUTHOR(S): Boquet, P. (1); Popoff, M. R. (1); Von Eichel-Streiber, C.; Giry, M. (1)  
CORPORATE SOURCE: (1) Unite Toxines Microbiennes, Inst. Pasteur, 28 rue du Docteur Roux, 75724 Paris, Cedex 15 France  
SOURCE: Microbial Ecology in Health and Disease, (1995) Vol. 8, No. 4, pp. 191.  
Meeting Info.: Workshop on Recent Advances in Clostridium difficile and its Toxins Tours, France May 4, 1995  
ISSN: 0891-060X.  
DOCUMENT TYPE: Conference  
LANGUAGE: English

L21 ANSWER 18 OF 18 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 14  
ACCESSION NUMBER: 1994:291810 CAPLUS  
DOCUMENT NUMBER: 120:291810  
TITLE: Clostridium difficile toxin B acts on the GTP-binding protein Rho  
AUTHOR(S): Just, Ingo; Fritz, Gerhard; Aktories, Klaus; Giry, Murielle; Popoff, Michel R.; Boquet, Patrice; Hegenbarth, Silke; von Eichel-Streiber, Christoph  
CORPORATE SOURCE: Inst. Pharmakol. Toxikol., Univ. Saarlandes, Homburg-Saar, D-66421, Germany  
SOURCE: J. Biol. Chem. (1994), 269(14), 10706-12  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
Searcher : Shears 308-4994

AB C. difficile toxin B exhibits cytotoxic activity that is characterized by the disruption of the microfilamental cytoskeleton. Here the authors studied whether the GTP-binding Rho protein, which reportedly participates in the regulation of the actin cytoskeleton, is involved in the toxin action. Toxin B treatment of Chinese hamster ovary cells reveals a time- and concn.-dependent decrease in the ADP-ribosylation of Rho by Clostridium botulinum C3 exoenzyme in the cell lystate. Disruption of the microfilament system induced by C. botulinum C2 toxin or cytochalasin D does not cause impaired ADP-ribosylation of Rho. Toxin B exhibits its effects on Rho not only in intact cells but also when added to cell lysates. Besides endogenous Rho, RhoA-glutathione S-transferase (Rho-GST) fusion protein added to cell lysate showed decreased ADP-ribosylation after toxin B treatment. Immunoblot anal. reveals identical amts. of Rho-GST and no change in mol. mass after toxin B treatment compared with controls. ADP-ribosylation of Rho-GST purified from toxin B-treated cell lystate is inhibited, indicating a modification of Rho itself. Finally, transfection of rhoA DNA under the control of a strong promoter into cells protects them from the activity of toxin B. Altogether, the data indicate that C. difficile toxin B acts directly or indirectly on Rho proteins to inhibit ADP-ribosylation and suggest that the cytotoxic effect of toxin B involves Rho.

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DOCUMENT TYPE: Journal  
LANGUAGE: English

AB In *S. cerevisiae*, the *ras*-related protein Rho1p is essentially the only target for ADP-ribosylation by exoenzyme C3 of *Clostridium botulinum*. Using C3 to detect Rho1p in subcellular fractions, Rho1p was found primarily in the 10,000 g pellet (P2) contg. large organelles; small amts. also were detected in the 100,000 g pellet (P3) and cytosol. When P2 organelles were sepd. in sucrose d. gradients, Rho1p comigrated with the Kex-2 activity, a late Golgi marker. Rho1p distribution was shifted from P2 to P3 in several mutants that accumulate post-Golgi vesicles. Rho1p comigrated with post-Golgi transport vesicles during fractionation of P3 organelles from wild-type or sec6 cells. Vesicles contg. Rho1p were of the same size but different d. than those bearing Sec4p, a *ras*-related protein located both on post-Golgi vesicles and the plasma membrane. Immunofluorescence microscopy detected Rho1p as a punctate pattern, with signal concd. towards the cell periphery and in the bud. Thus, in *S. cerevisiae* Rho1p resides primarily in the Golgi app. and also in vesicles that are likely to be early post-Golgi vesicles.

L17 ANSWER 21 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 12  
ACCESSION NUMBER: 1990:136524 CAPLUS  
DOCUMENT NUMBER: 112:136524  
TITLE: Multiple small molecular weight guanine nucleotide-binding proteins in human erythrocyte membranes

AUTHOR(S): Damonte, Gianluca; Sdraffa, Adina; Zocchi, Elena; Guida, Lucrezia; Polvani, Carolina; Tonetti, Michela; Benatti, Umberto; **Boquet, Patrice**; De Flora, Antonio

CORPORATE SOURCE: Dep. Biochem., Univ. Genoa, Genoa, 16132, Italy  
SOURCE: Biochem. Biophys. Res. Commun. (1990), 166(3), 1398-405  
CODEN: BBRCA9; ISSN: 0006-291X

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Native membranes from human erythrocytes contain the following G proteins which are ADP-ribosylated by a no. of bacterial toxins: Gi.alpha. and G0.alpha. (pertussis toxin), Gs.alpha. (cholera toxin), and 3 proteins of 27, 26, and 22 kDa (exoenzyme C3 from *Clostridium botulinum*). Three addnl. C3 substrates (18.5, 16.5, and 14.5 kDa) appeared in conditions of unrestrained proteolysis during hemolysis. SDS-PAGE sepn. of erythrocyte membrane proteins followed by electroblotting and incubation of nitrocellulose sheets with radiolabeled GTP revealed consistently 4 GTP-binding proteins with Mr values of 27, 26, 22, and 21 kDa. Although a 22-kDa protein was immunochem. identified as *ras* p21, the C3 substrate of 22 kDa is a different protein, probably identifiable with a rho gene

Searcher : Shears 308-4994

09/126816

product. Accordingly, at least 5 distinct small-mol.-wt. guanine nucleotide-binding proteins, whose functions are so far undetd., are present in native human erythrocyte membranes.

L17 ANSWER 22 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 13  
ACCESSION NUMBER: 1989:419628 CAPLUS  
DOCUMENT NUMBER: 111:19628  
TITLE: The mammalian G protein rhoC is ADP-ribosylated by Clostridium botulinum exoenzyme C3 and affects actin microfilaments in Vero cells  
AUTHOR(S): Chardin, P.; Boquet, P.; Madaule, P.; Popoff, M. R.; Rubin, E. J.; Gill, D. M.  
CORPORATE SOURCE: Fac. Med. Lariboisiere Saint-Louis, Paris, 75010, Fr.  
SOURCE: EMBO J. (1989), 8(4), 1087-92  
CODEN: EMJODG; ISSN: 0261-4189  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB C. botulinum C3 is a recently discovered exo-enzyme that ADP-ribosylates an eukaryotic GTP-binding protein in the ras superfamily. Bacterially-expressed product of the human rhoC gene is ADP-ribosylated by C3 and corresponds in size, charge and behavior to the dominant C3 substrate of eukaryotic cells. C3 treatment of Vero cells results in the disappearance of microfilaments and in actinomorphic shape changes without any apparent direct effect upon actin. Thus the ADP-ribosylation of a rho protein seems to be responsible for microfilament disassembly and the unmodified form of a rho protein may be involved in cytoskeletal control.

L17 ANSWER 23 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 14  
ACCESSION NUMBER: 1988:108713 CAPLUS  
DOCUMENT NUMBER: 108:108713  
TITLE: Functional modification of a 21-kilodalton G protein when ADP-ribosylated by exoenzyme C3 of Clostridium botulinum  
AUTHOR(S): Rubin, Eric J.; Gill, D. Michael; Boquet, Patrice; Popoff, Michel R.  
CORPORATE SOURCE: Sch. Med., Tufts Univ., Boston, MA, 02111, USA  
SOURCE: Mol. Cell. Biol. (1988), 8(1), 418-26  
CODEN: MCEBD4; ISSN: 0270-7306  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Exoenzyme C3 from C. botulinum types C and D specifically ADP-ribosylated a 21-kilodalton cellular protein, p21.bot. Guanyl nucleotides protected the substrate against denaturation, which implies that p21.bot is a G protein. When introduced into the interior of cells, purified exoenzyme C3 ADP-ribosylated intracellular p21.bot and changed its function. NIH 3T3, PC12, and  
Searcher : Shears 308-4994

09/126816

COUNTRY OF AUTHOR: FRANCE; INST PASTEUR, CNRS, UNITE TOXINES  
MICROBIENNES 557, F-75015 PARIS, FRANCE; IST SUPER  
SANITA, DEPT ULTRASTRUCT, I-00161 ROME, ITALY  
USA; JAPAN; FRANCE; ITALY  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF  
THE UNITED STATES OF AMERICA, (26 APR 1994) Vol. 91,  
No. 9, pp. 3814-3818.  
ISSN: 0027-8424.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 45

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Cytotoxic necrotizing factor type 2 (CNF2) produced by *Escherichia coli* strains isolated from intestinal and extraintestinal infections is a dermonecrotic toxin of 110 kDa. We cloned the CNF2 gene from a large plasmid carried by an *Escherichia coli* strain isolated from a lamb with septicemia. Hydropathy analysis of the deduced amino acid sequence revealed a largely hydrophilic protein with two potential hydrophobic transmembrane domains. The N-terminal half of CNF2 showed striking homology (27 % identity and 80 % conserved residues) to the N-terminal portion of *Pasteurella multocida* toxin. Methylamine protection experiments and immunofluorescence studies suggested that CNF2 enters the cytosol of the target cell through an acidic compartment and induces the reorganization of actin: into stress fibers. Since the formation of stress fibers in eukaryotic cells involves Rho proteins, we radiolabeled these small GTP-binding proteins from CNF2-treated and control cells with a Rho-specific ADP-ribosyltransferase. The [<sup>32</sup>P]ADP-ribosylated Rho proteins from CNF2-treated cells migrated slightly more slowly in SDS/PAGE than did the labeled proteins from the control cells. This shift in mobility of Rho proteins in SDS/PAGE was also observed when CNF2 and the RhoA protein were coexpressed in *E. coli*. We propose that Rho proteins are the targets of CNF2 in mammalian cells.

117 ANSWER 20 OF 24 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 11  
ACCESSION NUMBER: 1991:627970 CAPLUS  
DOCUMENT NUMBER: 115:227970  
TITLE: The small GTP-binding protein Rholp is localized  
on the Golgi apparatus and post-Golgi vesicles  
in *Saccharomyces cerevisiae*  
AUTHOR(S): McCaffrey, Mary; Johnson, Joni S.; Goud, Bruno;  
Myers, Alan M.; Rossier, Jean; Popoff, Michel  
R.; Madaule, Pascal; Boquet, Patrice  
CORPORATE SOURCE: Lab. Physiol. Nerveuse, CNRS, Gif-sur-Yvette,  
91198, Fr.  
SOURCE: J. Cell Biol. (1991), 115(2), 309-19  
CODEN: JCLBA3; ISSN: 0021-9525  
Searcher : Shears 308-4994